

Antifungal Effect of Mangosteen (*Garcinia mangostana*) and Pomegranate (*Punica granatum*) Extracts

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ABSTRACT

Medicinal plants serve as important raw materials in the development of new drugs. This study combined two extracts of mangosteen and pomegranate in different proportions and determined its effect against four fungi species (Candida albicans, Candida guilliermondii, Trichophyton mentagrophytes and Aspergillus niger). Experimental research utilizing randomized complete blocking design was used. There were 186 plates of which only 98 were valid. Treatments consisted of the different concentrations of the individual extract (100%, 90%, 80%, 70%, etc.) and combined fruit extract (90%:10%, 80%:20%, 70%:30%, etc. pomegranate:mangosteen). The zone of inhibition is the measure used in determining the growth inhibiting effect of the different treatments. Biosafety Clearance was complied. Results revealed that all ten concentrations of the mangosteen and the pomegranate extract possessed antifungal effect against Candida albicans and Candida guilliermondii. The 100 percent concentration demonstrated the largest zone of growth inhibition. None of the given concentrations of the extract demonstrated antifungal effect against Trichophyton mentagrophytes and Aspergillus niger. When both fruit extracts were combined to determine its synergistic effect, the 90 and 10 percent pomegranate: mangosteen combination exerted the best activity demonstrating a 33 mm zone of inhibition (ZOI) against Candida albicans which is comparable to the zone of inhibition exhibited by the Fluconazole against the same fungus. For Candida guilliermondii, all combinations were effective against the fungus. The combined extract is better than the pure ones. Researchers may try other possible methods of extraction, use other parts of the plants or solvents that can effectively extract active components.

Keywords: *fungi, optimum concentration, synergistic effect, zones of inhibition*

INTRODUCTION

The National Prevalence Survey of Fungal and Skin Diseases conducted by the Philippine Dermatologic Society shows that an average of 26.7 percent of Filipinos has significant dermatologic problems. Ranking fifth among the most common dermatologic problems (which is next to arthropod bites, scabies, acne vulgaris, and *Pityriasis versicolor*), is Dermatophytic Infection. It has a prevalence rate of 19.2 per 1000 population. When taken together with pityriasis versicolor, the superficial fungal infection becomes the most common dermatologic problem in the

country (42.6 per 1000 prevalence rate) making it a public health concern (Dayrit, et al., 2013).

Among the dermatophytic infections surveyed, *Tinea corporis* (ringworm) is the most common type of infection. Tropical conditions of high heat and humidity, overcrowding, poor sanitation, lack of fundamental health services, and malnutrition have contributed to the increasing number of people with these kinds of diseases. Among the people surveyed, only less than one percent consulted a physician. Instead, they relied on pieces of advice from relatives, neighbors, friends, or media, regarding various topical preparations, most of which are of no known value. The Department of Health, in line with the Philippine Drug Policy's call towards self-reliance in developing drug products from medicinal plants strongly advocated the use of scientifically tested and affordable medicinal plants.

Mangosteen is a tropical fruit that is widespread in countries in Southeast Asia due to its hot and humid climates. The fruit has dark purple color rind and measures about 2 to 3 inches in diameter. According to folk literature, the pericarp is used for healing since ancient times. It is used to prepare a hot beverage to relieve diarrhea, bladder infections, and gonorrhoea. It can also be in the form of an ointment applied to skin rashes. The pericarp has been documented in literature to possess xanthenes that included alpha-mangostin, beta-mangostin, garcinone B, and garcinone E. Aside from xanthenes, the tannins in the rind of the fruit are responsible for medicinal properties. Tannins have anti-inflammatory, antiseptic, and astringent properties. Mangosteen has also been found to have anti-inflammatory, antimicrobial, and antiseptic properties in test tube studies (Narasimhan, et al., 2017).

Mangosteen has high nutrients, strong antioxidant and shows great potential to prevent diseases. *Garcinia mangostana* is a natural source of dietary xanthenes and possesses over 30 different phenols. Its dark purple pericarp holds the highest level of xanthenes. However, among the xanthone derivatives, only 15% in mangosteen have been studied. Mangosteen, otherwise known as the "Queen of Fruits", has compounds alpha-mangostin, beta-mangostin, garcinone B and garcinone E, collectively called xanthenes. The rind of the fruit contains phytochemicals which discourage infestation by insects, fungi, plant viruses, and bacteria. Some phytochemicals are pigments giving the exocarp its purple color, including phenolic acids, also called phenols. The xanthenes of the mangosteen were tested to kill common fungi and demonstrated the capability of inhibiting their growth (Narasimhan, et al., 2017).

Pomegranate, on the other hand, is locally known as "Granada" in the Philippines. It is one of the many plant species that has many health benefits. It is commonly referred to as a super fruit due to its medicinal and nutritive values. It is responsible for lowering cholesterol levels, cardiac risk factors, and maintaining a

healthy prostate in males. Pomegranate is an excellent source of nutrients including vitamin C. Some of its active components are ellagic acid, ellagitannins, punicic acid, flavonoids, anthocyanidins, anthocyanins, estrogenic flavonols, and flavones. Different parts of the fruit have specific components. The fruit juice contains anthocyanins, glucose, ascorbic acid, ellagic acid, gallic acid, caffeic acid, catechin, quercetin, rutin, minerals, and amino acids. The seed contain punicic acid, pluellagic acid, fatty acids, and sterols. The leaves give tannins and flavones. The bark provides alkaloids. Flowers have gallic and ursolic acid. The pericarp contains punicalagin. The presence of these different compounds in the fruit make it an excellent raw material in the development of a drug.

Candida albicans is a fungus common among people that resulted in a history of overuse of antibiotics, steroidal medications, alcohol, and high sugar diets. It is generally the major cause of fungal infection worldwide. It is otherwise called monilia, a diploid fungus that grows both as yeast and filamentous cells. The fungus is normally present in different parts of the body but can be infective when it grows out of proportions inside the body. It is a natural flora of the mouth, but when there is immense number, especially in patients with compromised immune system, it can result in oral candidiasis or oral thrush. It is also a regular inhabitant of the vagina but in high numbers, may lead to vulvo-vaginal candidiasis, which is commonly known as yeast infection. The fungus also can enter the systemic circulation, and affect the throat, intestines, and heart valves. Infection of the esophagus can result in esophageal candidiasis. Untreated candidiasis may cause candidemia, where infection spread to the bloodstream and affect other organs. Ordinarily, *Candida* requires a moist, damaged skin for growth. The fungi rapidly colonize broken skin and intertriginous sites. Lowering of host resistance is necessary for *Candida* to invade host's deeper cutaneous tissues.

Another fungus employed in this study was *Candida guilliermondii*. On Sabouraud's Dextrose Agar, colonies are white to cream in color, smooth in consistency, and yeast-like in appearance. Microscopic appearance reveals round to oval-shaped, budding yeast-like cells or blastoconidia, 2.0-4.0 x 3.0-6.5 μm in size. The fungus can be isolated from numerous human infections, mostly of cutaneous origin. Systemic contaminations of *Candida guilliermondii* are rare.

Aside from the two *Candida* species stated above, another fungus utilized in the experiment was *Trichophyton mentagrophytes*. It is a specific type of fungus belonging to a larger group of fungi called the dermatophytes. This group of fungi produces a wide range of cutaneous diseases in both humans and animals. *T. mentagrophytes* has unique individuality with anthropophilic structure exhibiting thin aerial mycelium with plentiful spores. Colonies on Potato Dextrose Agar are powdery or granular. The most unique feature of this fungus is the production of tiny, drop-like aleuriospores assembled in grape-like clusters. The fungus can be isolated in the soil, the floor of swimming pools, hairs of wild and domesticated

animals, sandals, shower stalls and in between human toes without clinical manifestations. It has the ability to dissolve keratinous substances by both chemical and mechanical ways through the action of its keratinolytic enzymes. These enzymes are the key pathogenic factors of the fungus causing infections in both humans and animals.

The fourth fungus used in the study was *Aspergillus niger*. Colonies of the fungus on Czapek Dox Agar are typically leathery-like, rough and not glistening. They are cinnamon-buff to sand-brown in color with a yellow to deep dirty brown reverse. Conidial heads are condensed, elongated, and usually arranged in pairs. *Aspergillus niger* is the third widespread species associated with invasive pulmonary aspergillosis. It is the frequent causative agent of aspergilloma and otomycosis.

Although both mangosteen and pomegranate are found to have inhibitory activities against certain species of fungi, researchers continue testing the extracts of the different parts of the plants using different solvents and against more species of microorganisms to determine the fruits' maximum potential. In this particular study, the researcher tried another strategy – combining the two extracts of mangosteen and pomegranate in different proportions and determining its effect against four species (*Candida albicans*, *Candida guilliermondii*, *Trichophyton mentagrophytes* and *Aspergillus niger*) of fungi.

The conduct of the experiment was guided by the framework illustrated below:

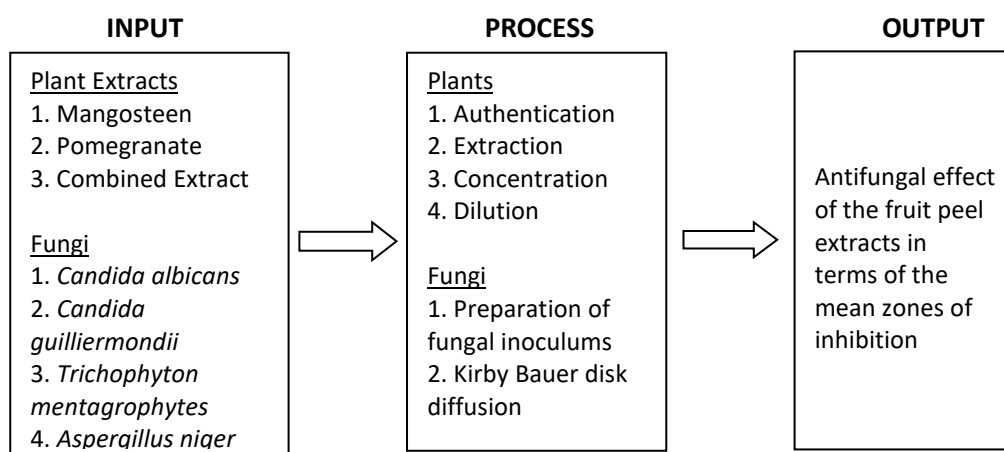


Figure 1. The Framework of the Study

MATERIALS AND METHODS

This study used experimental research utilizing randomized complete blocking design. The method involved the use of groups of fruits studied only once

but with succeeding treatments applied to determine the most and least efficacious of the trials. The pericarp ethanolic extracts obtained from the purple mangosteen and pomegranate were analyzed individually at different concentrations, and then mixed at different proportions to establish the synergistic effect of these extracts against the test fungi.

Plant Materials

The researcher used mature fruits of mangosteen and pomegranate in the study. The fruits were purchased in a local fruit stand. Voucher specimen numbers PLT-ID-CRPSD-140-18 and 141-18 have been deposited in the Bureau of Plant Industry, Department of Agriculture, Manila, the agency that verified and authenticated the fruit samples.

Specimen Processing

Both fruits underwent the same treatment. First, they were washed with tap water to remove all dirt and substances that may have adhered on the surface. The fruits were then unpeeled, and the pericarps were collected and cut into small pieces using a knife. They were then oven-dried at 45-50°C for 48-72 hours. The dried pericarps were homogenized into powder using an electrical blender.



Plate 1. Dried Mangosteen pericarps.



Plate 2. Dried Pomegranate pericarps.

Soxhlet Extraction (Reference: Royal Society of Biochemistry)

Twenty (20) grams of the pulverized sample of each fruit was subjected to Soxhlet extraction using 200 mL of the 95 percent ethanol. The extraction process was carried out for two hours. The resulting mixture was concentrated using the Rotavap to evaporate the alcohol until the mixture became syrupy in consistency. The temperature used was based on the boiling point of ethanol, which is 65 percent.

Preparation of the Culture Medium (Method by Clinical and Laboratory Standard Institute)

Mueller-Hinton agar supplemented with Glucose and Methylene Blue (MHA-GMB) was the culture medium used to demonstrate zones of inhibition of the fungi used in this study. It was prepared by mixing 58 grams of the MHA-GMB in one liter of distilled water and boiled to dissolve the medium completely. It was then autoclaved at 15 pounds per square inch (psi), 121°C for 15 minutes, allowed to cool at 45°C and dispensed in previously sterilized petridishes. Solidified culture media were stored inside the refrigerator until use.

Fungi Inoculum

Four fungal strains were utilized in the study. These were *Candida albicans*, *Candida guilliermondii*, *Trichophyton mentagrophytes*, and *Aspergillus niger*. These fungi were sourced-out from the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines, Los Baños, Laguna. From three to five days old subculture of each of the test fungi, conidia 3-5 colonies were isolated and transferred into a test tube containing 5mL NSS. The turbidity of the suspension was compared using 0.05 McFarland standard.

Preparation of the Mangosteen and Pomegranate Extract Concentrations

In the preparation of the individual extracts, ten (10) different concentrations were prepared for each of the fruit extracts using a spot plate. Each concavity of the spot plate was labeled M1 to M10 for the Mangosteen and P1 to P10 for the Pomegranate extract. M1/P1 represents 100 percent extract concentration; M2/P2 represents 90 percent; and so on. For M1/P1, 100µL of the 100 percent mangosteen/pomegranate extract was transferred. For M2/P2, 90µL of the 100 percent mangosteen/100 percent pomegranate extract and 10µL of distilled water were combined. For M3/P3, 80µL of the 100 percent mangosteen/100 percent pomegranate extract and 20µL of distilled water were combined, and so on.

In the preparation of the combined extract, each concavity of the spot plate was labeled MP1 to MP10. For MP1, 100µL of the 100 percent mangosteen with no pomegranate extract was transferred. For MP2, 90 µL of the 100 percent mangosteen extract and 10µL of the 100 percent pomegranate extract were combined. For MP3, 80µL of the 100 percent mangosteen and 20µL of 100 percent pomegranate extract were combined, and so on. Consequently, nine blank disks for every concentration per extract per test fungus (6mm diameter) were immersed into the different dilutions of the extracts and were allowed to dry inside the incubator maintained at 37 °C.



Plate 3. Preparation of the different extract concentrations – Pomegranate, Mangosteen, and the Combination of the Two Extracts.

Disc Diffusion Susceptibility Testing (Kirby Bauer Method)

This test was conducted at the Department of Microbiology, College of Public Health, University of the Philippines, Manila. Each of the prepared fungal inoculums was spread uniformly using a sterile cotton swab into the MHA-GMB agar plates. Then paper discs containing the different concentrations of the three extract preparations were distributed uniformly on the surface of the agar plates. Similarly, positive control and negative control disks were used side by side with the test disks. The control solutions used in the experiment were sterile distilled water (negative control) and 25 μ g Fluconazole (positive control).

The different plates were incubated at 25^oC-30^oC for 24 hours for *Candida albicans* and *Candida guilliermondii*, 48 hours for *Aspergillus niger* and 7 days for *Trichophyton mentagrophytes* (Standard Operating Procedure, Department of Medical Microbiology, College of Public Health, University of the Philippines, Manila). After incubation, the researcher measured the zones of inhibition using a Vernier caliper.

The following descriptive interpretation of the zones of growth inhibition was used in the interpretation of the result: 19mm and above – susceptible; 15-18mm – moderate (dose dependent); 14 and below – resistant. This interpretative rating is recommended by the Clinical Laboratory Standards Institute, the approved governing body that develops and publishes standards and guidelines in Microbiology.

The two statistical tools used in this study were the Mean and One-Way Analysis of Variance (ANOVA). ANOVA was used to analyze if the significant variations exist between group means at different concentrations.

Biosafety Clearance

The research proposal was reviewed and approved by the UNP Ethics Review Committee before it was implemented.

RESULTS AND DISCUSSION

Antifungal effect of the mangosteen extract

Table 1
Antifungal activity of the mangosteen extract

Concentration of the Mangosteen Extract and Controls	Against <i>Candida albicans</i>		Against <i>Candida guilliermondii</i>		Against <i>T. mentagrophytes</i>		Against <i>Aspergillus niger</i>	
	Mean Zone of Inhibition	Descriptive Rating	Mean Zone of Inhibition	Descriptive Rating	Mean Zone of Inhibition	Descriptive Rating	Mean Zone of Inhibition	Descriptive Rating
25µg Fluconazole	38mm	Susceptible	32mm	Susceptible	30mm	Susceptible	30mm	Susceptible
100% Mangosteen	25mm	Susceptible	24mm	Susceptible	6mm	Resistant	6mm	Resistant
90% Mangosteen	23mm	Susceptible	21mm	Susceptible	6mm	Resistant	6mm	Resistant
80% Mangosteen	21mm	Susceptible	20mm	Susceptible	6mm	Resistant	6mm	Resistant
70% Mangosteen	21mm	Susceptible	20mm	Susceptible	6mm	Resistant	6mm	Resistant
60% Mangosteen	21mm	Susceptible	18mm	Susceptible (Dose Dependent)	6mm	Resistant	6mm	Resistant
50% Mangosteen	20mm	Susceptible	18mm	Susceptible (Dose Dependent)	6mm	Resistant	6mm	Resistant
40% Mangosteen	16mm	Susceptible (Dose Dependent)	12mm	Resistant	6mm	Resistant	6mm	Resistant
30% Mangosteen	16mm	Susceptible (Dose Dependent)	11mm	Resistant	6mm	Resistant	6mm	Resistant
20% Mangosteen	10mm	Resistant	8mm	Resistant	6mm	Resistant	6mm	Resistant
10% Mangosteen	9mm	Resistant	7mm	Resistant	6mm	Resistant	6mm	Resistant
Distilled Water	6mm	Resistant	6mm	Resistant	6mm	Resistant	6mm	Resistant

Descriptive Rating Legend:
 (Clinical Laboratory Standards Institute)

19mm and above Susceptible
 15-18mm Susceptible dose dependent (DD)
 14 and below Resistant

The antifungal activity of the Mangosteen extract in terms of the Zones of Inhibition (ZOI) against *C. albicans* and *C. guilliermondii* shows a direct association between the concentration of the extract and the ZOI it produces. The result further means that as the concentration of the extract increases, the ZOI also increases. It is remarkable to observe that *Candida albicans* is susceptible to the extract starting at 50 percent concentration while the latter is susceptible only starting at 70 percent concentration. In contrast with the two *Candida* species, the Mangosteen extract exhibited no ZOI against *T. mentagrophytes* and *A. niger* in all concentrations (6mm

is the measurement of the paper disk). Only the 25µg Fluconazole (Positive Control) exhibited ZOI against the two fungi.

The antifungal activity of Mangosteen was due to the presence of the different phytochemicals in the fruit. One of the many active constituents present in the fruit belongs to a group of xanthone derivatives such as alpha, beta, gamma mangostin, garcinone, mangostanol, and gartinin. Among these, alpha-mangostin has shown to exert the most effective antibacterial activity by several studies. These substances were classified as polyphenols (Kaomongloglit et al., 2013).

According to the study conducted by Kaomongloglit et al. (2013) the result showed that alpha-mangostin was effective against *C. albicans*. The killing action of alpha-mangostin was said to be more effective than Clotrimazole and Nystatin, which are the common antifungal drugs used. On the other hand, Ragasa et al. (2015) conducted an experiment using the ethyl acetate extract of the freeze-dried pericarp of *Garcinia mangostana L.* At 30 µg, the extract exhibited a strong inhibitory activity against *S. aureus*, moderate activity against *C. albicans* and low activity against *B. subtilis*, *E. coli*, *P. aeruginosa*, *T. mentagrophytes*, and *A. niger*.

Based on the study done by Chaitra et al. (2015) the presence of polyphenols in fruits or various plants was the one responsible for its antifungal activity. Polyphenols was believed to impede the growth of many fungi because it was proven to be toxic to endospore by destroying the cell wall while the presence of tannins prevents the growth of fungi by precipitating the microbial proteins (Chaitra et al. 2015).

Table 2 presents the antifungal activity of the Pomegranate extract in terms of the Zones of Inhibition (ZOI) against *C. albicans* and *C. guilliermondii*. It exhibited a wide ZOI ranging from 18 mm to 30 mm. Surprisingly, even the 10 percent concentration produced a moderate inhibitory activity (18 mm). This result implies that the Pomegranate extract has strong antifungal activity against *C. albicans* and *Candida guilliermondii*.

Antifungal Effect of the Pomegranate Extract

Table 2
Antifungal activity of the pomegranate extract

Concentration of the Pomegranate Extract and Controls	Against <i>Candida albicans</i>		Against <i>Candida guilliermondii</i>		Against <i>T. mentarophytes</i>		Against <i>A.niger</i>	
	Mean Zone of Inhibition	Descriptive Rating	Mean Zone of Inhibition	Descriptive Rating	Mean Zone of Inhibition	Descriptive Rating	Mean Zone of Inhibition	Descriptive Rating
25µg Fluconazole	38mm	Susceptible	32mm	Susceptible	30mm	Susceptible	30mm	Susceptible
0% Pomegranate	30mm	Susceptible	29mm	Susceptible	6mm	Resistant	6mm	Resistant
90% Pomegranate	29mm	Susceptible	28mm	Susceptible	6mm	Resistant	6mm	Resistant
80% Pomegranate	29mm	Susceptible	28mm	Susceptible	6mm	Resistant	6mm	Resistant
70% Pomegranate	28mm	Susceptible	28mm	Susceptible	6mm	Resistant	6mm	Resistant
60% Pomegranate	27mm	Susceptible	28mm	Susceptible	6mm	Resistant	6mm	Resistant
50% Pomegranate	26mm	Susceptible	26mm	Susceptible	6mm	Resistant	6mm	Resistant
40% Pomegranate	25mm	Susceptible	23mm	Susceptible	6mm	Resistant	6mm	Resistant
30% Pomegranate	24mm	Susceptible	21mm	Susceptible	6mm	Resistant	6mm	Resistant
20% Pomegranate	22mm	Susceptible	19mm	Susceptible	6mm	Resistant	6mm	Resistant
10% Pomegranate	18mm	Susceptible (Dose Dependent)	18mm	Susceptible (Dose Dependent)	6mm	Resistant	6mm	Resistant
Distilled Water	6mm	Resistant	6mm	Resistant	6mm	Resistant	6mm	Resistant

Descriptive Rating Legend:
 (Clinical Laboratory Standards Institute)

19mm and above	Susceptible
15-18mm	Susceptible dose dependent (DD)
14 and below	Resistant

The presence of various phytochemicals, namely hydrolysable tannins and polyphenolic compounds are contributory to the antifungal activity of Pomegranate extract. The tannins, such as punicalin, punicalagin, gallagylidilacton, pedunculagin, tellimagrandin I, and cortilagin were believed to be responsible for the antimicrobial activity of the fruit extract (Foss et al., 2014). Likewise, the same study, observed the enhanced activity of the isolated compound punicalagin against *C. albicans* and *C. parapsilosis*, indicating the substance as a potent antifungal agent, without elucidating the mechanism of action. Siqueira et al. (2011) assign to the tannins the ability to inhibit the growth of the yeast, *Candida* species, due to their activity in the cell, specifically in the cell membrane, precipitating proteins. Also, data obtained in

the research done by Alexandre et al. (2019) showed the antimicrobial capacity of pomegranate extract against yeast cells of *Candida* genus with a minimum inhibitory concentration of 125 µg/mL. According to the researcher, the bioactive compounds which are tannins present in the pericarp and peel showed antifungal activity. These bioactive compounds could be accountable for changes in cell morphology, preventing growth, generating various materials, and bursting the cells.

Duman et al. (2014) investigated the antimicrobial activity of six varieties of *Punica* correlating the responses to the phytonutrient properties, such as total phenolic and anthocyanin compounds, and found positive results regarding the inhibition of Gram positive and Gram-negative microorganisms and *Candida albicans*, linking these findings to the antioxidant capacity of this plant.

Contrary to the two *Candida* species, the pomegranate extract is ineffective against *T. mentagrophytes* and *A. niger*. This result ran contrary to the study of Dahham et al. (2014). In Dahham's research, the methanolic extracts of pomegranate exhibited a high antifungal activity to *A. niger* with ZOI values between 8 and 23 mm. The rind extract exhibited a high inhibitory effect with a ZOI of 23 mm, then the juice with a ZOI of 20 mm, and the white seed with a ZOI of 8 mm. Similarly, Foss et al., (2014) demonstrated through in vitro experiment that the crude extract of pomegranate fruit peel has antifungal activity against *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum canis*, and *M. gypseum*, with a minimum inhibitory concentration of 125 µg/mL and 250 µg/mL respectively for each genus. The conflicting result may be due to the differences in the extracting agent. Dahham used methanol while this study utilized ethanol as the extracting agent. Another factor that may be considered is the age of the fungi. Older fungi have thicker chitin (cell wall) than younger ones. If the chitin is thick, the harder for the extract to penetrate thereby less susceptible.

Antifungal Effect of the Combined Mangosteen - Pomegranate Extract

When the Mangosteen and Pomegranate extracts were combined and prepared at different proportions then tested against *C. albicans*, it produced wider ZOI as when tested against the fungus separately. The ZOI ranged from 21 mm to 33 mm. The best combination that gave the widest ZOI is ten (10) parts Mangosteen and 90 parts Pomegranate, giving a mean ZOI of 33 mm.

Table 3
Antifungal activity of the combined mangosteen - pomegranate extract

Proportion of the Mangosteen to Pomegranate in μL	Against <i>Candida albicans</i>		Against <i>Candida guilliermondii</i>		Against <i>T. mentagrophytes</i>		Against <i>A. niger</i>	
	Mean Zone of Inhibition	Descriptive Rating	Mean Zone of Inhibition	Descriptive Rating	Mean Zone of Inhibition	Descriptive Rating	Mean Zone of Inhibition	Descriptive Rating
25 μg Fluconazole	38mm	Susceptible	32mm	Susceptible	30mm	Susceptible	30mm	Susceptible
10% : 90% (M:P)	33mm	Susceptible	32mm	Susceptible	6mm	Resistant	6mm	Resistant
20% : 80% (M:P)	31mm	Susceptible	30mm	Susceptible	6mm	Resistant	6mm	Resistant
30% : 70% (M:P)	30mm	Susceptible	29mm	Susceptible	6mm	Resistant	6mm	Resistant
40% : 60% (M:P)	30mm	Susceptible	29mm	Susceptible	6mm	Resistant	6mm	Resistant
50% : 50% (M:P)	28mm	Susceptible	26mm	Susceptible	6mm	Resistant	6mm	Resistant
60% : 40% (M:P)	26mm	Susceptible	25mm	Susceptible	6mm	Resistant	6mm	Resistant
70% : 30% (M:P)	25mm	Susceptible	24mm	Susceptible	6mm	Resistant	6mm	Resistant
80% : 20% (M:P)	23mm	Susceptible	22mm	Susceptible	6mm	Resistant	6mm	Resistant
90% : 10% (M:P)	21mm	Susceptible	20mm	Susceptible	6mm	Resistant	6mm	Resistant
Distilled Water	6mm	Resistant	6mm	Resistant	6mm	Resistant	6mm	Resistant

M : P

Descriptive Rating Legend:
 (Clinical Laboratory Standards Institute)

Mangosteen : Pomegranate

19mm and above Susceptible
 15-18mm Susceptible dose dependent (DD)
 14 and below Resistant

When the same solution was tested against *C. guilliermondii*, it produced an almost the same ZOI with the ten (10) parts Mangosteen extract, and 90 parts Pomegranate extract exhibiting the best antifungal activity. This proportion of the combined extract is as efficient as the 25 μg Fluconazole giving the same ZOI. However, when the same solution was tested against different species of fungi (*T. mentagrophytes* and *A. niger*), no ZOI were observed. It means that the combined extract had no antifungal activity against these organisms.

The synergistic antifungal activities of the combined extracts of mangosteen and pomegranate were due to the presence of various phytochemical compounds found in both fruits which were tannins and polyphenolic compounds (Chaitra et al., 2015). The findings noted herein were also similar to the studies made by Caprinella et al. (2013) which stated that from the seed kernels of *Melia azedarach L.*, a substance named hydroxycoumarin scopoletin was isolated from which three other compounds, vanillin, 4-hydroxy-3-methoxycinnamaldehyde, and pinosresinol were isolated. Guided fractionation on Thin Layer Chromatography using *Fusarium verticillioides* as test organism led to the isolation of vanillin, which exhibited a

minimum inhibitory concentration (MIC) of 1.50 mg/mL in the microbroth dilution method. Despite its own weak activity, when the Coumarin combined with the vanillin, an improved antifungal effect was observed. A similar result exhibited when the synthetic antifungal agents, Mancozeb and Carboxin were combined with the compounds stated, in which cases it became possible to decrease the effective concentration of these commercial compounds by up to 2.5 and 3 percent, respectively. These support the findings of this study that the combined effect of two active compounds will result to better ZOI compared to the pure extracts of the mangosteen and pomegranate.

The Optimum Concentration of Each Extract That Could Inhibit the Growth of the Four Fungi

Among the ten (10) concentrations of the mangosteen extract and ten (10) concentrations of the pomegranate extract, both had an optimum concentration of 100%. On the other hand, among the nine (9) proportions of the combined extract, the optimum level is at 90 percent pomegranate and 10 percent mangosteen against *C. albicans* and *C. guilliermondii*.

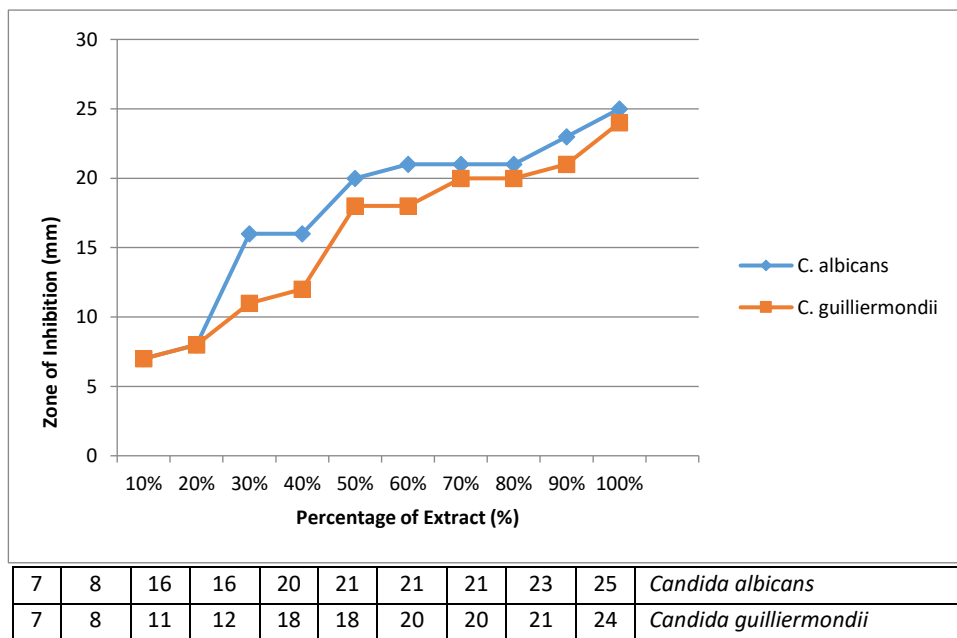


Figure 2. Mangosteen extract against *Candida albicans* and *Candida guilliermondii*

Figure 2 shows the optimum concentration of the mangosteen extract that is most effective against *C. albicans* and *C. guilliermondii*. The 100 percent extract concentration produced the highest antifungal activity with a ZOI of 25 mm, and 24 mm respectively.

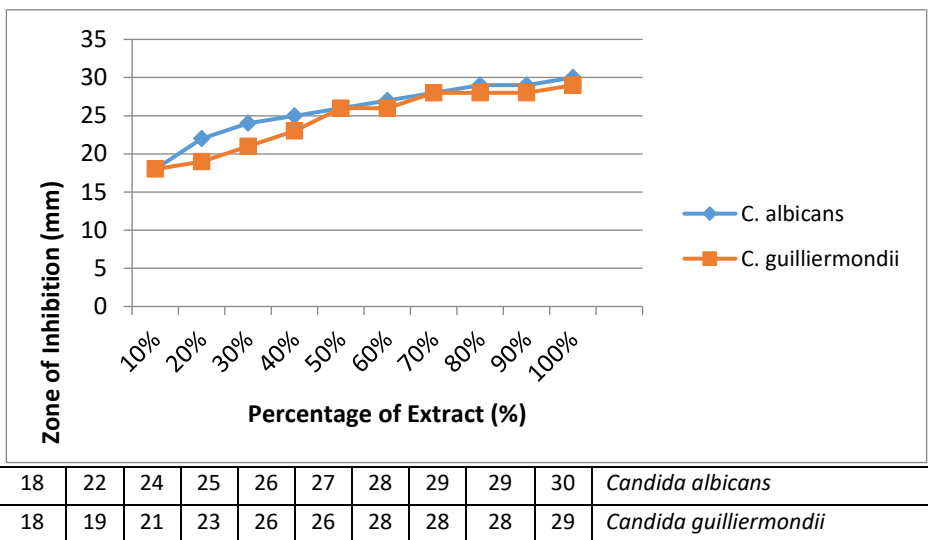


Figure 3. Pomegranate extract against *Candida albicans* and *Candida guilliermondii*

Figure 3 presents the optimum concentration of the pomegranate extract that is most effective against *C. albicans* and *C. guilliermondii*. The 100 percent extract concentration produced the highest antifungal activity with a ZOI of 30 mm, and 29 mm, respectively.

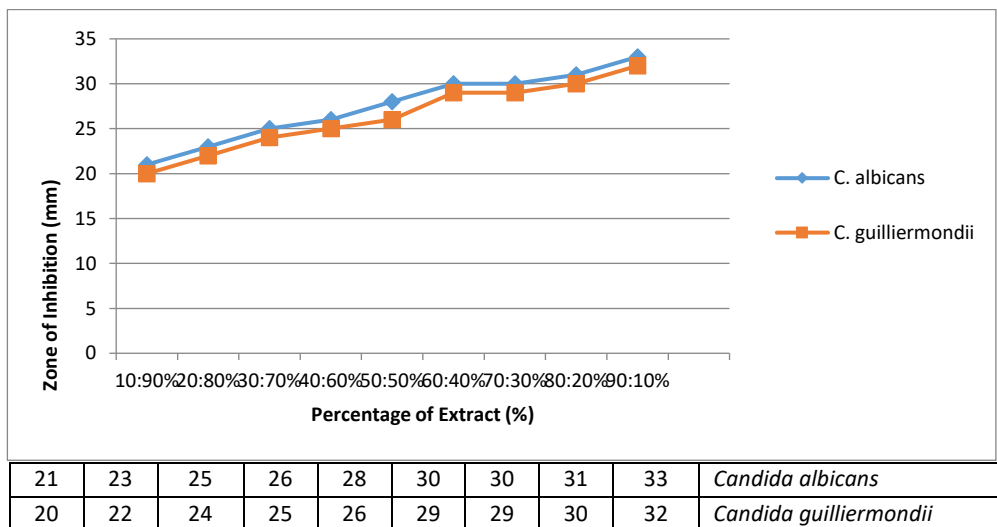


Figure 4. Combined mangosteen and pomegranate extract against *Candida albicans* and *Candida guilliermondii*

The optimum concentration for the combined extract is shown in Figure 4 in which the 90 percent pomegranate and 10 percent mangosteen concentration produced the highest ZOI against the two *Candida* species with inhibition zones of 33 mm and 32 mm respectively.

In general, the higher the concentration of the mangosteen extract and pomegranate extract, the higher is the ZOI. The peak of inhibition was at 100% of the mangosteen extract and pomegranate extract used individually and 10:90 (mangosteen:pomegranate) extract used in combination against *C. albicans* and *C. guilliermondii*. Among the three different extracts used, it was the mangosteen extract that produced the least ZOI against the two *Candida* species followed by the pomegranate extract. The combined extracts of the two fruits used in the study yielded the greatest ZOI.

Significant Difference in the Antifungal Activities of the Different Concentrations of the Pure Extracts of Mangosteen and Pomegranate

The Two-Way Analysis of Variance was used to determine if a significant difference exists in the antifungal activities among the pure extracts of mangosteen and pomegranate. Both extracts recorded remarkable ZOI for *C. albicans* and *C. guilliermondii*. As evidenced by the obtained F-stat and F-critical value, the result suggested that there is a significant difference between the mangosteen and the pomegranate extracts in different concentrations in terms of its antifungal activity for the two *Candida* species tested. Since the result of the F-test stated that there was a significant difference between the four different variables tested, therefore the null hypothesis is rejected.

Significant Difference in the Antifungal Activity of the Combined Extracts in the Different Concentrations

As shown by the obtained F stat and F critical value, the combined extracts and *C. albicans* have a computed F of 22.36 meaning that there was a significant difference between the two variables. The null hypothesis, therefore, is rejected. Based on the result obtained, only the optimum concentration of 90 percent

Table 4
Variations in antifungal activity of each of the mangosteen and pomegranate extracts in different concentrations

Variables	Computed F	Analysis	Decision
Mangosteen Extract and <i>Candida albicans</i>	5.391	Significant	Reject Ho
Mangosteen Extract and <i>Candida guilliermondii</i>	19.13	Significant	Reject Ho
Pomegranate Extract and <i>Candida albicans</i>	16.38	Significant	Reject Ho
Pomegranate Extract and <i>Candida guilliermondii</i>	6.42	Significant	Reject Ho
Fluconazole Solution and <i>Candida albicans</i>	25.40	Significant	Reject Ho
Fluconazole Extract and <i>Candida guilliermondii</i>	24.98	Significant	Reject Ho

Critical F at df 2/9 and 0.05 level of significance is 4.26

Note: *T. mentagrophytes* and *A. niger* have no antifungal activity, hence, they are not included in finding the significant difference.

Table 5
Variations in antifungal activity of the combined extracts of mangosteen and pomegranate in different proportions

Variables	Computed F	Analysis	Decision
Combined Extracts and <i>Candida albicans</i>	22.36	Significant	Reject Ho
Combined Extracts and <i>Candida guilliermondii</i>	3.34	Not Significant	Accept Ho

Critical F at df 2/8 and 0.05 level of significance to 4.46.

pomegranate extract and 10 percent mangosteen extract is recommended. On the other hand, for the variables combined extracts and *C. guilliermondii*, it has a computed F of 3.34, meaning that there was no significant difference among the variables tested. The null hypothesis, therefore, is accepted. Thus, all the nine (9) different concentrations of the combined extracts prepared against *C. guilliermondii* can be used.

CONCLUSIONS

All ethanolic extracts used in this study exhibited an antifungal effect against *C. albicans* and *C. guilliermondii* as manifested by their remarkable ZOI. All extracts were observed to be ineffective against *T. mentagrophytes* and *A. niger*. The combined extract of 90 percent pomegranate and 10 percent mangosteen exhibited a synergistic antifungal effect against *C. albicans* and in all nine preparations against *C. guilliermondii*. The presence of phytochemicals in the mangosteen and pomegranate extracts including phenols, tannins, and flavonoids as the main active constituents were responsible for these antifungal effects.

RECOMMENDATIONS

The researcher recommends future studies dwelling on this topic to try other methods of extraction that will not subject the extracts to extreme temperature; to utilize another fruit to be paired with mangosteen or pomegranate or to use other fruit combinations which can also exhibit a synergized antifungal activity to different fungi; to use another part of the fruit rather than the pericarp alone to assess the antifungal property of the combined extracts; to isolate xanthenes and punicalagin using High Performance Liquid Chromatography (HPLC) or Mass Spectroscopy for more accurate isolation of their concentration; to test the antifungal property of the extracts with other fungi species; and to employ another solvent for extraction that could effectively extract all of the active substances from the plant samples.

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