

CHAPTER 2

REVIEW OF RELATED LITERATURE

2.1 *Candida albicans*

2.1.1 Introduction and Taxonomy

Candida albicans is a species of Gram-positive diploid fungus which is a form of yeast that is commonly present as normal flora on the skin and in mucous membranes such as the vagina, mouth, or rectum. *Candida albicans* is derived from the word *candidatus*, a custom in ancient Rome for a public office to wear white and *albico* meaning “to be white”. It is sometimes called as *Monilia* and can cause opportunistic oral and genital infections in humans. The fungus can affect the throat, intestines, and heart valves when it enters the bloodstream and becomes an infectious agent when there are changes in the body environment that allow it to grow out of control (Healthscout, 2009; Simi, 2010).

Candida albicans causes no ill effects in 80% of the human population under normal circumstances but an overgrowth can result in candidiasis. Candidiasis is often found in immunocompromised individuals such as HIV-positive patients, and patients undergoing chemotherapy or organ/bone transplantation. The usual unicellular yeast-like form of *Candida albicans* responds to environmental changes and switches into an invasive and multicellular filamentous form to infect the host cell. (Ryan and Ray, 2004).



Figure 1 *Candida albicans* growing on an agar plate

Scientific classification:

Kingdom	: Fungi
Phylum	: Ascomycota
Subphylum	: Saccharomycotina
Class	: Saccharomycetes
Order	: Saccharomycetales
Family	: Saccharomycetaceae
Genus	: <i>Candida</i>
Species	: <i>Candida albicans</i>
Binomial name	: <i>Candida albicans</i>

Synonyms : *Candida stellatoidea*, *Oidium albicans* (Dan, 1952)

2.1.2 Morphology and Identification

Candida appear as oval, budding yeast cells which are 3-6 μm in size when grown in culture or tissue. Pseudohyphae are formed when the buds

continue to grow but fail to detach, producing chains of elongated cells that are pinched or constricted at the septations between cells (Brooks et al., 2007).

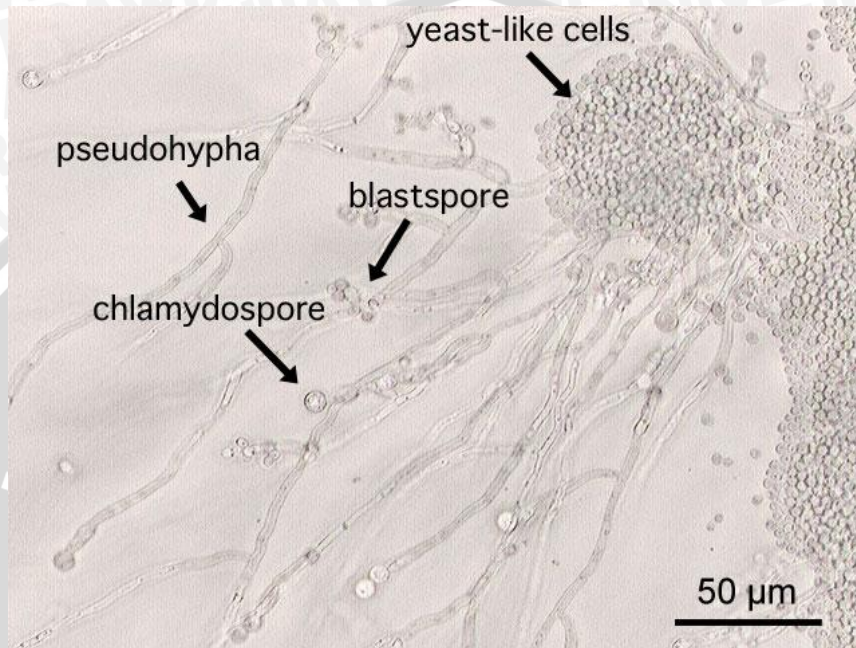


Figure 2 Various morphological forms of *Candida albicans* (Calderone and Fonzi, 2001)

Candida albicans is unlike any other species of *Candida* in which it can be dimorphic meaning that besides yeasts and pseudohyphae, it can also produce true hyphae. On agar media or within 24 hours at 37 °C or room temperature, *Candida* species produce soft, cream-colored colonies with a yeasty odor. Pseudohyphae appear as submerged growth below the agar surface (Brooks et al., 2007).

Two simple morphologic tests are used to distinguish *C. albicans*, which is the most common pathogen, from other species of *Candida*: After incubation in serum for about 90 minutes at 37 °C, yeast cells of *C. albicans* will begin to form true hyphae or germ tubes while on nutritionally deficient media, *C. albicans*

produces large, spherical chlamyospores. Sugar fermentation and assimilation tests are used to identify and speciate the more common *Candida* isolates, such as *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. kefyr*, *C. krusei*, and *C. lusitanae*; *C. glabrata* is unique among these pathogens because it produces only yeast cells and no pseudohyphal forms (Brooks et al., 2007).

2.1.3 Antigenic Structure

The usage of adsorbed antisera has defined two serotypes of *C. albicans* which are A and B. Many other antigens have also been characterized, that include secreted proteases, an immunodominant enolase, and heat shock proteins (Brooks et al., 2007).

2.2 Candidiasis

2.2.1 Definition

Candidiasis is an infection caused by a group of microscopic fungi or yeast. It is commonly referred to as *yeast infection*, and also technically known as *candidosis*, *moniliasis*, and *oidiomycosis*. There are more than 20 species of *Candida*, the most common being *Candida albicans*. These fungi live on all surfaces of our body and under certain conditions; they become so numerous that they cause infections, particularly in warm and moist areas. Some examples of such infections are vaginal yeast infection, thrush, skin and diaper rash, and nailbed infection (Stoppler, 2009).

Candidal infections usually occur in warm moist body areas, such as underarms. Yeast usually cannot penetrate the skin, but any breakdown or cuts in the skin may allow this organism to enter. In babies, it is typically the mouth

and diaper areas that are commonly affected. In adults, it is usually as oral yeast infection but they can also have yeast infections around dentures, under the breasts and lower abdomen, nailbeds, and beneath other skin folds. Most of these candidal infections are superficial and can be treated easily. In rare cases, the yeast infection may spread throughout the body and in systemic candidal disease; up to 75% of people may die. There have been cases where even common mouth and vaginal yeast infections can cause critical illness and can be more resistant to normal treatment. Candidal infections that return may be a sign of a more serious disease such as diabetes, leukemia, or AIDS (Stoppler, 2009).

2.2.2 Epidemiology

In the United States, *Candida* species are the most common cause of fungal infection in immunocompromised persons. Oropharyngeal colonization can be found in 30-55% of healthy young adults, and *Candida* species may be detected in 40-65% of normal fecal flora. Vulvovaginal candidiasis can occur at least once in the lifetime of three out of every four women worldwide. More than 90% of persons infected with HIV who are not receiving highly active antiretroviral therapy eventually develop oropharyngeal candidiasis, and 10% eventually develop at least one episode of esophageal candidiasis. In persons with systemic infections, *Candida* species are now the fourth most commonly isolated pathogens from blood cultures. Clinical and autopsy studies have confirmed the marked increase in the incidence of disseminated candidiasis, reflecting a parallel increase in the frequency of candidemia (Hidalgo and Vazquez, 2010).

A similar increase in cases of mucocutaneous and systemic candidiasis/candidemia has been observed worldwide. In fact, throughout the

world, *Candida* species have replaced *Cryptococcus* species as the most common fungal pathogens affecting immunocompromised hosts (Hidalgo and Vazquez, 2010).

Most candidal infections are mucocutaneous which do not cause mortality. However, in patients with advanced immunodeficiency due to HIV infection, these mucosal infections do not respond to antifungal therapy and may lead to severe oropharyngeal and esophageal candidiasis, that initiates a vicious cycle of poor oral intake, malnutrition, wasting, and early death (Hidalgo and Vazquez, 2010).

2.2.3 Pathogenesis and Pathology

Yeast infections are the second most common reason for vaginal burning, itching and discharge. They are found in the vagina of most women and can overgrow if the environment in the vagina changes due to antibiotic and steroid use, which is the common reason for yeast overgrowth, along with pregnancy, menstruation, sperm, diabetes, and oral contraceptives. The usage of detergents douches or internal disturbances (hormonal or physiological) can modify the normal vaginal flora and result in an overgrowth. Candidiasis is more common after menopause (Stoppler, 2009).

A weak or low immunity or metabolic illnesses such as diabetes are main predisposing factors for candidiasis as *Candida* is opportunistic. The diseases or conditions linked to candidiasis include AIDS, mononucleosis, cancer treatments, steroids, stress, and nutrient deficiency (Stoppler, 2009).

Candida may build up in an area due to frequent use of strong antibiotics, which kill the bacteria which are the normal flora that normally keeps it under control (Stoppler, 2009).

The use of implantable devices such as urinary catheter and IV ports also provide access for the yeast to enter the body. IV drug addicts using dirty needles may inject the yeast directly into their blood stream or deep tissues (Stoppler, 2009).

Sexual intercourse with an infected individual, low immunity, antibiotics, and diabetes are the common causes for *penile candidiasis*. Male genital yeast infection is less common but infection from direct contact via sexual intercourse with an infected partner is not uncommon (James and Berger, 2006).

Superficial candidiasis which can be cutaneous or mucosal, is caused by an increase in the number of *Candida* and damage to the skin or epithelium that permits local invasion by the yeasts and pseudohyphae. Systemic candidiasis occurs when *Candida* enters the bloodstream and the phagocytic host defenses are unable to contain the growth and dissemination of the yeasts. *Candida* can infect the kidney, attach to prosthetic heart valves, or produce candidal infections almost anywhere when in the bloodstream. The local histology of cutaneous or mucocutaneous lesions is characterized by inflammatory reactions varying from pyogenic abscesses to chronic granulomas. The lesions contain abundant budding yeast cells and pseudohyphae. A huge increase of *Candida* population in the intestinal tract often occurs after the administration of oral antibacterial antibiotics which can then enter the circulation by crossing the intestinal mucosa (Brooks et al., 2007).

2.2.4 Immunology

The basis of resistance to candidiasis is complex and not completely understood. Cell-mediated immune responses, especially CD4 cells, are

important in controlling mucocutaneous candidiasis, and the neutrophil is probably crucial to systemic candidiasis (Brooks et al., 2007).

2.2.5 Clinical Manifestations

Clinical manifestations of candidiasis differ depending on the location of the infection. Signs and symptoms of a vaginal yeast infection are pain with sexual intercourse, burning with urination, and a whitish-gray cottage cheese-like discharge that typically itches and irritates the vagina and surrounding tissues (Stoppler, 2009).

In infants and adults, a candidal infection can appear as:

- Oral candidiasis (thrush) – thick, white lacy patches on top of a red base forms on the tongue, palate or elsewhere inside the mouth. These patches sometimes look like milk curds and if the white plaques are wiped away, the underlying tissue may bleed. Accompanying symptoms include odynophagia or pain when swallowing.
- Superficial candidal skin infections – red flat rash with sharp scalloped edges with “satellite lesions”. These rashes may cause itching or pain (WebMD, 2005).

In immunocompromised people, candidal infections can affect various internal organs and cause pain or dysfunction of the organ. About 85% of people with AIDS contract a yeast infection called esophagitis in their upper gastrointestinal tract. This infection is similar to thrush but extends down the mouth and esophagus to the stomach. *Candida* esophagitis can cause painful

ulcers throughout the gastrointestinal system, making it too painful to swallow even liquids (Stoppler, 2009).

Candidemia produces symptoms such as feeling sick with or without fever. If the infection spreads to the brain, acute changes in mental function or behavior can be observed (Stoppler, 2009).

Symptoms of infection of the male genitalia include red patchy sores near the head of the penis or on the foreskin, severe itching, or a burning sensation. Candidiasis of the penis can also have a white discharge, although uncommon (David *et al*, 1997).

2.2.6 Classification of Candidiasis

Candidal infections are divided into two categories which are mucocutaneous candidiasis and invasive systemic candidiasis. Mucocutaneous candidiasis is described as infection of the mucous membranes and skin, whereby the infections are more superficial and on the outer parts of the body while invasive systemic candidiasis refers to infections of the organs of the body and the bloodstream (White, 2000).

Types of mucocutaneous candidiasis:

- Oral candidiasis (thrush)
- Perlèche (angular cheilitis)
- Candidal vulvovaginitis
- Candidal intertrigo
- Diaper candidiasis
- Congenital cutaneous candidiasis
- Perianal candidiasis

- Candidal paronychia
- Erosion interdigitalis blastomycetica
- Chronic mucocutaneous candidiasis
- Antibiotic candidiasis
- Candidal folliculitis
- Candidal balanitis
- Mammary candidiasis

Invasive systemic candidiasis describes severe and invasive disorders that include candidemia, disseminated candidiasis, deep organ involvement, endocarditis, endophthalmitis, and meningitis. It can occur throughout the entire body and can be life-threatening. The blood, brain, eye, kidney, and heart are most frequently affected, but the candidal fungus also can grow in the lungs, liver and spleen (WebMD, 2005).

2.2.7 Diagnostic Laboratory Tests

Medical professionals may use two primary methods to diagnose yeast infections: microscopic examination and culturing (Brooks *et al.*, 2007).

2.2.7.1 Specimens

Specimens can be obtained from swabs and scrapings from superficial lesions, blood, spinal fluid, tissue biopsies, urine, exudates, and material from removed intravenous catheters (Brooks *et al.*, 2007).

2.2.7.2 Microscopic Examination

Gram-stained smears are used to examine the samples obtained which tissue biopsies, centrifuged spinal fluid, and other specimens for pseudohyphae and budding cells. Skin or nail scrapings are first placed in a drop of 10% potassium hydroxide (KOH) and calcofluor. The KOH dissolves the skin cells but leaves the *Candida* cells intact, permitting visualization of pseudohyphae and budding cells (Brooks *et al.*, 2007).

2.2.7.3 Culture

To culture *Candida*, a sterile swab is first rubbed in the infected skin surface and then streaked on a Sabouraud agar plate as the medium for culture. The culture is incubated at 37°C for several days, to allow development of yeast or bacterial colonies. The characteristics such as morphology and colour of the colonies may allow initial diagnosis of the organism that is causing the disease symptoms. Contaminated Foley catheters may lead to “false-positive” urine cultures. Sputum cultures have no value because *Candida* species are part of the oral flora (Odds, 1987).

Sabouraud dextrose agar, cornmeal agar, and the eosin methylene blue agar medium can also be used for the culture of *Candida albicans*. On Sabouraud dextrose agar, soft cream-colored colonies of 0.5-1.5mm diameter appear with a yeasty odor. Cultures on the cornmeal agar medium at a temperature below 26°C form resting cells called chlamydo spores. Within 24-48 hours on eosin methylene blue agar at high carbon dioxide concentrations, the yeast forms a characteristic growth resembling spider legs or a Casuarina tree (Andrianto, 2007).

2.2.8 Treatment for Candidiasis

Candida species are frequently part of the human body's normal flora.

Treatment with antibiotics can lead to eliminating the yeast's natural competitors for resources, and increase the severity of the condition (Shiefer, 1997).

In clinical settings, candidiasis is commonly treated with antimycotics – the antifungal drugs commonly used to treat candidiasis are topical clotrimazole, topical nystatin, and topical ketoconazole. Thrush and other mucocutaneous forms of candidiasis are usually treated with topical nystatin or oral ketoconazole or fluconazole. Systemic candidiasis is treated with amphotericin B, sometimes in conjunction with oral flucytosine, fluconazole, or caspofungin. The clearing of cutaneous lesions is accelerated by eliminating contributing factors such as excessive moisture or anti-bacterial drugs. Chronic mucocutaneous candidiasis responds well to oral ketoconazole and other azoles, but patients have a genetic cellular immune defect and often require life-long treatment (Moosa et al., 2004; Brooks *et al.*, 2007).

It is often difficult to establish an early diagnosis of systemic candidiasis because the clinical signs are not definitive, and cultures are often negative. In addition, there is no established prophylactic regimen for those at risk. Treatment with an azole or with a short course of low-dose amphotericin B is often indicated for febrile or debilitated patients who are immunocompromised and do not respond to antibacterial therapy (Brooks *et al.*, 2007).

2.3 *Annona muricata* L.

2.3.1 Taxonomy



Figure 3 *Annona muricata* leaves

Scientific Classification:

Kingdom	: Plantae
Order	: Magnoliales
Family	: Annonaceae
Genus	: <i>Annona</i> L.
Species	: <i>Annona muricata</i>
Binomial name	: <i>Annona muricata</i> L.

Common names : *Graviola*, soursop, *guanábana*, *guanábano*, *guanavana*, *guanaba*, *corossol épineux*, *huanaba*, *toge-banreisi*, *durian benggala*, *nangka blanda*, *cachiman épineux*, *sirsak* (Family Content, 2006; Taylor, 2002)

2.3.2 Geographic Distribution

Annona muricata is widely grown and naturalized in the tropical areas of America and Western Africa where they can be found in Bermudas, Bahamas and all Western Indies starting from Southern Mexico to Peru and Argentina. *Annona muricata* is also introduced to areas such as Southeast China to Australia and the warm lowlands of both Eastern and Western Africa. It is a fruit commonly found in Malaysia and other parts of Southeast Asia which includes Indonesia. This species of plant prefers warm and humid climates and grows in well-drained soils. It grows well in slightly acidic soil pH of 5.5 to 6.5 with sand, slimy, clayey, and sandstone soils but can tolerate poor soils. The *Annona muricata* fruit is often cultivated in low forests in the tropics around the world while its flowers grow during the whole year. (Family Content, 2006)

2.3.3 Plant Description

Annona muricata plant is a low-branching and bushy tree which is slender due to its overturned limbs and can reach 7.5 to 9.0m. It has evergreen leaves which are alternate, smooth, glossy, dark green on the upper surface and lighter beneath. The leaves can be either oblong, elliptic or narrowbovate and is pointed at both ends. It is usually 6.25 to 20.0cm long and 2.50-6.25cm wide. The short stalked flowers of *Annona muricata* are born singly and emerge anywhere on the trunk, branches or twigs. It is 4 to 5cm long and has 3 fleshy, slightly spreading outer petals which are yellow green while the 3 close-set inner petals are pale-yellow. (Morton, 1987)

Annona muricata fruit has a shape which varies from oval to heart-shape and can be irregular, lopsided or curved because of improper carper

development or insect injury with sizes ranging from 10 to 30cm long, up to 15cm wide and weighing up to 4.5 to 6.8kg. The fruit has a reticulated, leathery-appearing but tender, inedible, bitter skin with “spines” which are stubby or more elongated and curved which are soft and pliable and breaks off easily when the fruit is fully ripe. The skin of the fruit is dark-green when immature and becomes slightly yellowish-green while inner surface of the skin is cream-colored and granular and separates easily from the snow-white flesh of the core. The close-packed segments of the core are mostly seedless but in each fertile segment, it contains a single oval, smooth, hard, black seed which is 1.25 to 2.0cm long in which a large fruit maybe contain a few dozen to 200 or even more seeds. (Morton, 1987)



Figure 4 The soursop flower



Figure 5 The soursop fruit

2.3.4 Historical or Traditional Uses of Soursop Leaf

The soursop leaves are used in many civilizations for different uses. One of them as a leaf decoction against head louse and bed bugs which can also be used as a sedative, antispasmodic, hypotensor and nervine (to tone up nerves and stimulate its action). In the Ecuadorian province of Esmeraldas, the leaf

decoction is used as an analgesic and antispasmodic while in Africa it is used for children in case of fever in which they also use it for gentle bathing. A decoction of tender shoots or leaves of the soursop plant is used in some parts of the Western Indies as a remedy for gallbladder diseases, as well as for colds, catarrh, diarrhea, dysentery and indigestion. It is also said to 'cool the blood' and able stop vomits and help during childbirth. The decoction can also be use as wet compresses on inflammation and swelled feet. (Family Content, 2006)

The leaves are also used as to make tea for a number of different uses. The native tribes in Guyana prepare a leaf tea and use it as a sedative and heart to tonic. In the Peruvian Andes, it is used against inflammation of the mucous membranes, such as that produced during catarrh while in the Peruvian Amazonia, it is for diabetes as well as a sedative and antispasmodic. In the Brazillian Amazonia, it is used for liver diseases, whereas oil extracted from the leaves and unripe fruits are mixed with olive oil and used externally for neuralgia, rheumatism, and arthritic pains. In British Guyana, the leaves in water are used to sober a drunk while a leaf tea or tea infusion is used in Hollander Antilles to produce a profound overnight sleep. (Family Content, 2006)

Chewed leaves of the soursop plant mixed with saliva are used to make the swelling disappear on incisions after surgery without producing a scar while mashed leaves are used as cataplasm to alleviate eczemas and other skin disorders as well as rheumatism. The syrup from tender leaves is poured for use on skin eruptions. (Family Content, 2006)

2.3.5 Cultivation and Uses

Annona muricata L. is cultivated in several tropical countries mainly for its fruit. The trees require much heat, humidity and water and do not survive at temperatures below 0° C. The plant can grow under direct sunlight or slight shadow and proliferates from sea level to a little more than 1000 m asl, particularly in humid regions of tropical countries. As the plant roots are superficial, it does not require deep soils and do not tolerate floodable soil.

(Family Content, 2006)

2.3.5.1 Ethnomedical Uses

The leaves of *Annona muricata* have a huge variety of uses. It is used as a decoction for head lice and bed bugs, as an analgesic and antispasmodic in some places as well as a remedy for gallbladder disease, colds, catarrh, diarrhea, dysentery and indigestion. (Family Content, 2006; Taylor, 2002)

Annona muricata is also reported to have anticancer properties where its leaves and stems have been found to be cytotoxic against cancer cells. (Family Content, 2006)

2.3.6 Constituents of *Annona muricata* Leaves

The main constituents of *Annona muricata* L. are Annonaceous acetogenins which include annocatalin, annohexocin, annomonicin, annomuricatin A & B, annomuricin A thru E, annonacin and annonacinone. Other notable chemicals are gentisic acid and condensed tannins. (Taylor, 2002)

Gentisic acid is a phenolic acid and derivative of benzoic acid which is known to have antifungal properties found in the leaves. (Kim et al, 2007; Taylor, 2002; Pubchem, 2012; Iqbal et al, 2010)

Condensed tannins or proanthocyanidins are phenolic compounds that precipitate proteins and are made from flavonoid units. (Arthur et al, 2011; Cannas, 2013)

2.3.7 Antifungal Properties of *Annona muricata*

Annonaceous acetogenins are natural products of annonaceous plants which are very potent inhibitors of the NADH-ubiquinone reductase (Complex I) activity of mammalian mitochondria. *Candida albicans* mitochondria share similarities with mammalian mitochondria and therefore, are also affected in the same way. The inhibition of Complex I could induce a state similar to enzyme dysfunction leading to increased reactive oxidant species (ROS), loss of chronological aging and programmed cell death (PCD). (Li et al, 2011; Yamaguchi et al., 1971; Zafra-Polo et al., 1996; Degli et al., 1994)

Gentisic acid (2,5-dihydroxybenzoic acid) is a derivative of benzoic acid and a type of phenolic acid which is a metabolite of aspirin/salicylic acid found in the leaves of the soursop. It becomes antifungal through the disruption of cellular redox homeostasis of cellular glutathione resulting in cellular GSH (reduced glutathione)/GSSG (oxidized glutathione) imbalances. Glutathione is suggested to be an essential metabolite in *C. albicans* and depletion of glutathione activates the redox signalling of the cells to undergo apoptosis. (Taylor, 2002; Kim et al, 2007; Baek, 2004; Franco, 2012; Cidlowski, 2012, Iqbal et al, 2010)

Condensed tannins are known to have antifungal properties and work by inhibiting the synthesis of chitin which is an important component of fungal cell wall besides glucans, mannans and glycoproteins, causing loss of cell wall integrity. (Arthur *et al*, 2011; Hwang *et al*. 2011; Ishida *et al.*, 2006, Bowman and Free, 2006)

2.4 Antifungal Sensitivity Identification

There are two methods which can be used to identify antimicrobial activity which are the disk diffusion method and the dilution method. The method which was chosen for this experiment was the tube dilution method.

2.4.1 Dilution Method

The dilution method can be done using a liquid media or broth in either a test tube or using compact agar on an agar plate and are known as the tube dilution test and agar dilution test respectively.

The tube dilution method uses a series of test tubes filled with liquid media and a certain amount of microbes that will be tested. Each test tube is filled with serially diluted antimicrobial dilutions and incubated at 37°C for 18-24 hours and its turbidity was observed. The test tube which has a translucent mixture indicating no microbial growth and also the lowest concentration for it to occur is the Minimum Inhibitory Concentration (MIC). All the tubes with translucent mixture are streaked on compact agar medium and incubated at 37°C for 18-24 hours. The agar medium was then observed for colonial growths and the agar medium with no colonial growth and lowest concentration is the Minimal Fungicidal Concentration (MFC).

The agar dilution method uses compact agar in which the antimicrobial solution is mixed with agar media when it is still liquid but not too hot and let to solidify. After the agar solidifies, the microbe is streaked and incubated for 24 hours at 35°C. The concentration of microbe and antimicrobial solution was monitored and the concentration with the least colonies of microbe which is less than three colonies is the Minimal Inhibition Concentration (MIC).

