EVALUATION OF ANTIMICROBIAL ACTIVITY OF *Aegle marmelos* (L.) Corrêa AND Cassia *alata* L. AGAINSTS PATHOGENIC MICROORGANISMS

By

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Chair : Associate Professor Nur Ain Izzati binti Mohd Zainudin, PhD
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Traditional medicine makes use of several plant species, including *Aegle marmelos* and *Cassia alata*, to cure a wide range of illnesses. The introduction of novel antibiotic-resistant bacteria and the spread of existing ones are major problems for public health around the world. The primary reason for conducting this research is to determine the efficacy of aqueous and methanol extracts of unripe fruit, *A. marmelos* leaves, and *C. alata* leaves against various microorganisms. Cold methanol and water extraction was used to extract the leaves and unripe fruit of *A. marmelos* and *C.* alata. Pathogenic bacteria (*Streptococcus agalactiae*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and yeasts (*Candida kruse*i and *Candida parapsilosis*) were tested for their susceptibility to the plant extracts using disc diffusion and agar well diffusion methods. Microdilution was used to establish the MIC and MFC, or minimal inhibitory and minimum fungicidal concentrations, respectively. Scanning electron microscopy (SEM) was utilised to examine the cellular modification induced by active plant extracts. The antifungal activity of the methanol extract of the fruit leaves of *A. marmelos* and *C. alata* was much higher than that of the water extract, which was ineffective against both *C. parapsilosis* and *C. krusei*. In contrast, the disc diffusion method did not detect any antibacterial activity from the methanol or aqueous extract. The minimum inhibitory concentration (MIC) for both A. marmelos leaf and C. alata unripe fruit against C. krusei and *C. parapsilosis* was found to be 75 mg/ml. However, the MIC for *C. parapsilosis* was reported to be 37.5 mg/ml. The MFC results for *C. krusei* and *C. parapsilosis* on *A. marmelos* leaf and unripe fruit and *C. alata* leaf all indicated 100% mortality at a concentration of 150 mg/ml. Fungistatic activity was demonstrated by the methanolic extract's minimal inhibitory and minimal fungigenic concentrations, respectively. The fungicidal and fungistatic properties of C. alata and *A. marmelos* leaves were demonstrated against *C. parapsilosis* and *C. krusei*. Under a scanning electron microscope (SEM), the effects of *A. marmelos* and *C. alata* methanol extract on *C. parapsilosis* and *C. krusei* were seen to cause a wide range of morphologic changes, such as wrinkles, breaks, bumps, holes in the cell wall, ruffles, and a raisin-like appearance. Based on these findings, *A. marmelos* and *C. alata* could serve as promising novel antifungal agent sources.

Keywords: *Aegle marmelos*, *Cassia alata*, MIC, MFC, SEM

SDG: SDG 15- Life on land

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENILAIAN AKTIVITI ANTIMIKROB OLEH *Aegle marmelos* (L.) Corrêa DAN *Cassiaalata* L. TERHADAP MIKROORGANISMA PATOGENIK

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Aegle marmelos (L.) Corrêa dan *Cassia alata* Linn adalah spesies tumbuhan yang digunakan dalam perubatan tradisional dalam rawatan pelbagai penyakit. Disebabkan perkembangan pesat rintangan mikrob terhadap antibiotik dan kemunculan strain baru telah membebankan kesihatan komuniti secara global. Oleh itu, objektif utama kajian ini adalah untuk menilai aktiviti antimikrob bagi ekstrak akueus dan metanol daripada buah muda dan daun *A. marmelos*, serta daun *C. alata*. Ekstrak daun dan buah muda *A. marmelos* dan daun *C. alata* telah disediakan melalui pengekstrakan sejuk menggunakan pelarut metanol dan air. Aktiviti antimikrob bagi ekstrak tumbuhan telah diuji ke atas bakteria patogen (*Streptococcus agalactiae*, Escherichia coli dan *Pseudomonas aeruginosa*) dan yis (*Candida krusei* dan *Candida parapsilosis*) menggunakan teknik resapan cakera dan telaga agar. Kepekatan perencatan minimum (KPM) dan kepekatan fungisida minimum (KFM) ditentukan dengan kaedah pencairan mikro. Perubahan selular yang disebabkan oleh ekstrak aktif telah diperhatikan dan dinilai menggunakan mikroskop elektron pengimbasan (SEM). Ekstrak metanol daun dan buah muda *A. marmelos* dan daun *C. alata* mempunyai aktiviti antikulat yang ketara terhadap *C. parapsilosis* dan *C. krusei* berbanding akueus yang tidak berkesan terhadap kedua-dua patogen. Sebaliknya, metanol dan ekstrak akueus tidak mendedahkan aktiviti antibakteria dalam kaedah resapan cakera. Nilai kepekatan perencatan minimum (KPM) daun, buah muda *A. marmelos* dan *C. alata* terhadap *C. krusei* dan adalah 75 mg/ml manakala daun *A. marmelos* mempunyai nilai kepekatan perencatan minima 37.5 mg/ml terhadap *C. parapsilosis*. Hasil kepekatan fungisida minimum (KFM) bagi *C. krusei* dan *C. parapsilosis* adalah 150 mg/ml bagi daun,buah muda *A. marmelos* dan daun *C. alata*. KPM dan KFM ekstrak methanol menunjukkan bahawa buah muda *A. marmelos* bertindak sebagai agen fungistatik. Daun *A. marmelos* dan *C. alata* berkesan sebagai agen fungistatik dan fungisida terhadap *C. krusei* dan *C. parapsilosis*. Pemerhatian mikroskop elektron pengimbasan (SEM) ekstrak metanol *A. marmelos* dan *C. alata* pada *C. parapsilosis* dan *C. krusei* menunjukkan pelbagai peringkat perubahan morfologi seperti bentuk tidak sekata dengan kedutan, pecah, bergelombang, kerosakan dinding sel yang berlubang, kecacatan dinding sel, renyuk, dan rupa seperti kismis. Hasil kajian menunjukkan bahawa ekstrak metanol *A. marmelos* dan *C. alata* berpotensi menjadi sumber agen antikulat yang baru.

Kata kunci : *Aegle marmelos*, *Cassia alata*, KPM, KFM, SEM

SDG: SDG 15- Life on land

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
MIC	Minimum Inhibitory Concentration
MFC	Minimum Fungicididal Concentration
mg/ml	Miligram per mililitre
µg/mL	Microgram per mililitre
mm	Milimeter
CFU/mL	Colony forming unit per mililtire
MTT	3-bis (2-methoxy-4-nitro- 5-sulphophenyl)-2H-tetrazolium-5-carboxanilide (MTT)
DMSO	Dimethyl Sufoxide
SEM	Scanning Electron Microscope
SE	Standard Error
Ca^{2+}	Calcium
TLR4	Toll-like receptor 4
SDA	Sabouraud Dextrose Agar
MH	Mueller Hinton Agar
ITZ	Itraconazole
CVC	Central Venous Catherer
ICU	Intensive care unit
MDR	Mutlt Drug Resistant
r.p.m	Radius per minute
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

1.1 Background of study

Many cultures have traditional uses for medicinal plants as the era of alternative medicine and holistic health approaches. Furthermore, these natural resources don't have to worry about the negative fallout that can result from man-made synthetic substances. Analysis of the potential antibacterial properties of many plant extracts and therapeutic ointments has found organic effects (Correia et al., 2016). Plant extracts' antimicrobial properties have served as the foundation for a wide variety of uses, including raw and processed food safety, pharmaceuticals, complementary and alternative medicine, and conventional therapies (Mahlo et al., 2016). Eco-friendly and bio-compatible plant-based products have recently received a lot of attention for the prevention and treatment of many human ailments (Sivanathan & Elamaran, 2013). Yuan et al. (2016) found that in both Asia and Africa, around 80% of the population relies on medicinal spices alone or in combination with traditional medicine. Two thousand kinds of medicinal plants are thought to exist in Peninsular Malaysia, with participation from a wide range of ethnic groups (Sabran etal., 2016). Medicinal plant use is based on "common sense," "perception," and "custom practise" derived from socioreligious convictions that have been passed down from generation to generation (Mohamad et al., 2019).

Plants also contain bioactive combinations that have been shown to be antimicrobial whether used singly or in combination with other antimicrobial drugs (Hu et al., 2020). There are less negative effects on animals, humans, and the environment when these mixes are used in traditional medicine (Ncube et al., 2012; Saha & Dasgupta, 2005), hence they are widely used as a crude extract for natural remedies. *Cassia alata* Linn and *Aegle marmelos (*L) Corrêa were used in this research as potential options for combating fungal and bacterial infections.

In addition to its more common names, the *Aegle marmelos* tree is also known as sripal or bilwa in Sanskrit, vilvam in Tamil, and stone apple or bael in English or Pokok Maja Batu in Malay. In addition to its native range in India, *A. marmelos* can also be found in a number of other countries and territories, including China, Nepal, Ceylon, Myanmar, Pakistan, Bangladesh, Nepal, Vietnam, Laos, Cambodia, Thailand, Indonesia, Malaysia, Tibet, Java, the Philippines, and Fiji (Yadav et al., 2015). The tree thrives in well-drained soil and can reach a height of 12-15 m in spite of the harsh and arid climate. The leaflets of its leaves range in size from three to five ovals and are pointy and shallowly serrated. The flowers can be seen in clusters all along the young branches, and they have a pleasant scent (Baliga et al., 2011). The tree is also said to have produced its first fruit in 1941 in Northern Malaysia (Sharma et al., 2007).

The *A. marmelos* plant, including its stem, bark, root, leaves, and fruits, has been used as a natural medicine in India's Ayurvedic, Unani, and Siddha medical systems (Raja et al., 2011). Aegelin, skimmianine, lupeol, cineol, citral, marmesinin, marmelide, cuminaldehyde, eugenol, -sitosterol, flavone, glycoside, and marmeline are just few of the bioactive chemicals isolated from *A. marmelos* leaf, fruits, bark, and root (Monika et al., 2023) Therapeutic qualities such as anti-diabetic, anti-ulcer, anti-oxidant, anti-malarial, anti-inflammatory, anti-cancer, anti-hyperlipidaemic, and anti-bacterial and anti-viral activities have been attributed to their extracts and used in traditional medicine (Somu et al., 2019).

Common names for the bush *Cassia alata* [synonym: Senna alata (L.) Roxb.] include Candle, Dadmardan, and Semaiagathi in India; the Roman Candle tree in Fiji; Gelenggan in Malaysia; and Ketepeng Badak in Indonesia (Alioes & Kartika, 2019). For optimal results, a small amount of lime is sometimes combined with the concentrate when the plant is used to treat ringworm infection and parasitic skin disease in Malaysia and Nigeria (Owoyale et al., 2006; Ibrahim & Osman, 1995). It is used to treat a wide variety of illnesses in India and South America, including skin disorders, asthma, snake bites, fever, digestive problems, and even heart diseases (Yeong et al., 2017).

According to Somchit et al. (2003),*Cassia alata* found in these regions Southeast Asia, Fiji, Northern Australia, Africa, and Latin America. The anti-inflammatory, analgesic, antifungal, hypoglycemic, diuretic, and wound-healing properties of a *C. alata* leaf extract have been well documented (Mohideen et al., 2005). There are some flavonoids and anthraquinones in the ethanol extract of *C. alata* leaves (Kumar et al., 2016). While chrysophanol, emodin, and rhein all have antibacterial properties (Wuthi-Udomler et al., 2010), rhein is the most potent.

1.2 Problem statement and research questions

Plants have long been a go-to source for restorative drugs, playing an important role in healthcare and providing a wealth of potential novel antimicrobial drugs for use against bacteria and other drug-resistant microbes. It is possible that the new antibacterial agents will be cloaked in natural ointments and extracts from medicinal plants (Sabo & Knezevic, 2019). However, antimicrobial drug supplies are limited, and widespread use of these drugs for treatment has contributed to the rise of drug-resistant microorganisms. This research aimed to answer the following analytical questions about the antimicrobial and antifungal efficacy of *A. marmelos* and *C. alata* extracts against infectious microorganisms: i. two, how effective are *A. marmelos* and C. alata extracts against bacteria and fungus, as measured by their minimal inhibitory, bactericidal, and fungicidal concentrations? Extracts of *A. marmelos* and *C. alata* are hypothesised to be effective against *Candida* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus agalactiae*, and to be useful in the treatment of related disorders. This research will help determine whether or not plant extracts may be used as an alternative to readily available conventional medication for treating microbial infections.

1.3 Aims and importance of study

Pathogenic microorganisms, such as fungus and bacteria, are becoming increasingly resistant to antibacterial treatments in the coming years (Raoufi & Shafaghat, 2018). Presently, there is a serious threat to the clinical application of anti-infection drugs and the effective treatment of bacterial infections due to the proliferation of bacteria that have developed resistance to numerous types of antibiotics (Mandrone et al., 2019). Eighty percent of people in non-industrialized countries rely on plant medicine as their primary source of healthcare (Dulger, 2009). Half of all newly synthesised particles derived from natural sources have demonstrated their value in the development of drugs for the treatment of infectious disorders (Baliga et al., 2011).

Traditional plant medicines for bacterial and infectious diseases may provide inspiration for the creation of new, effective natural antibiotics (Murugan & Kamaraj, 2018). Tannins, terpenoids, alkaloids, flavonoids, and glycosides are just some of the many optional metabolites found in plants that have been shown to exhibit antibacterial activities when tested in vitro (Dubey et al., 2019). In addition, they are effective against bacteria, fungi, viruses, and protozoa, making them useful for treating a wide variety of skin conditions, including dermatitis, burns, diarrhoea, fever, wounds, cuts, sores, hacking, and localised skin swellings (Pesewu et al., 2008). Because of the plant's origin, these factors compelled botanists and pharmaceutical companies to go elsewhere for treatments (Al Farraj et al., 2020). These explanations suggest that traditional medicine has merit when compared to modern medicine (Mahlo et al., 2016).

1.4 Research objectives

The objectives of this study are:

i. To evaluate the antimicrobial activities of *A. marmelos* and *C. alata* on *Candida* krusei (ATCC 6258), *Candida parapsilosis* (ATCC 22019), *Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus agalactiae.*

ii. To examine the morphological changes of selected pathogens treated with plant extract under the scanning electron microscope (SEM).

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