# PHYTOCHEMICALS ANALYSIS, ANTIOXIDANT, AND ANTIBACTERIAL ACTIVITIES OF *Salvinia molesta* D.S.Mitch (Salviniaceae)

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For my beloved *abah*, Md. Salleh bin Abdullah,
For my lovely sisters (Nur Asshura & Nur Ashira Solehah)

In the name of Allah, The Most Gracious and The Most Merciful,
This study dedicated to all of you.
May Allah grant these beautiful souls Jannah.

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# ABSTRACT

The occurrence of invasive alien aquatic plant species in a habitat is recognized as the second largest threat to biodiversity after habitat loss. *Salvinia molesta* D.S.Mitch is classified as an obnoxious and noxious invasive aquatic plant in Malaysia, and it has been listed as the world's worst invasive alien species in Global Invasive Species Database (GISD) since 2013. The main goal of this study was to gain knowledge on the potential medicinal values of *S. molesta* extracts (aqueous and ethanol). Thus, the quantification of phytochemical contents, antioxidant, and antibacterial activities of *S. molesta* extracts were determined. The aqueous and ethanol extracts of *S. molesta* were prepared through the maceration technique. These extracts then were quantified for their total phenolic, flavonoid, tannin, alkaloid, and saponin contents following the standard methods. Besides, the antioxidant activity of the extracts was evaluated by DPPH, ABTS, and FRAP assays. Moreover, both aqueous and ethanol extracts of *S. molesta* were examined for their antibacterial activity by disc-diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) methods against selected bacterial strains, such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. As a result, higher contents of phytochemicals were recorded in the ethanol extract at tannin followed by phenolic, saponin, flavonoid, and alkaloids. Thereupon, the extract obtained from ethanol exhibited high antioxidant capacity based on DPPH, ABTS, and FRAP assays. Moreover, the ethanol extract expressed high antibacterial activity against *B. cereus*, followed by *E. coli*, *S. aureus*, and *P. aeruginosa* as tested using disc-diffusion method. The MIC and MBC against *S. molesta* extracts also revealed that the ethanol extract can inhibit growth and kill all the bacteria strains tested. The study highlighted that ethanol extract exhibited higher phytochemical content, significant antioxidant, and antibacterial properties than aqueous extract.

## ABSTRAK

Kewujudan spesies tumbuhan akuatik asing yang invasif dalam sesebuah habitat diiktiraf sebagai ancaman kedua terbesar kepada kepelbagaian biologi, selepas kehilangan habitat. *Salvinia molesta* D.S.Mitch diklasifikasikan sebagai tumbuhan akuatik invasif yang kurang menyenangkan dan berbahaya di Malaysia, dan ia telah disenaraikan sebagai spesies asing invasif terburuk di dunia dalam *Global Invasive Species Database* (GISD) sejak 2013. Tujuan utama kajian ini adalah untuk mendapatkan maklumat berkaitan potensi perubatan bagi ekstrak akueus dan etanol *S. molesta*. Oleh itu, kandungan fitokimia, aktiviti antioksidan dan antibakteria bagi ekstrak *S. molesta* telah ditentukan. Ekstrak akueus dan etanol *S. molesta* telah dihasilkan melalui teknik maserasi. Jumlah kandungan fenolik, flavonoid, tanin, alkaloid, dan saponin bagi kedua-dua ekstrak dikira secara kuantitatif mengikut kaedah piawai. Selain itu, aktiviti antioksidan ekstrak dinilai melalui ujian DPPH, ABTS, dan FRAP. Di samping itu, aktiviti antibakteria kedua-dua ekstrak akueus dan etanol *S. molesta* juga diuji melalui kaedah resapan cakera, kepekatan perencatan minimum (MIC), dan kepekatan membunuh bakteria minimum (MBC) terhadap beberapa bakteria terpilih, seperti *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, dan *Pseudomonas aeruginosa*. Hasil dapatan kandungan fitokikmia yang lebih tinggi direkodkan dalam ekstrak etanol pada tanin diikuti oleh fenolik, saponin, flavonoid, dan alkaloid. Ekstrak daripada etanol menunjukkan kapasiti antioksidan yang tinggi berdasarkan ujain DPPH, ABTS, dan FRAP. Selain itu, ekstark etanol *S. molesta* menunjukkan aktiviti antibakteria tertinggi terhdap *B. cereus*, diikuti oleh *E. coli*, *S. aureus*, dan *P. aeruginosa* apabila diuji menggunakan kaedah resapan cakera. MIC dan MBC terhadap ekstrak *S. molesta* juga mendedahkan bahawa ekstrak etanol boleh menghalang pertumbuhan bakteria pada dan membunuh semua bakteria yang diuji. Kajian ini menujukkan bahawa ekstrak etanol mempunyai kandungan fitokimia yang tinggi, dan mempamerkan sifat antioksidan dan antibakteria yang ketara berbanding ekstrak akueus.

# CONTENTS

## LIST OF TABLES

# LIST OF FIGURES

## LIST OF SYMBOLS AND ABBREVIATIONS

| | | |
|---|---|---|
| *ºC* | - | Degree Celsius |
| *%* | - | Percentage |
| *c* | - | Concentration |
| *g* | - | Gram |
| *m* | - | Mass |
| *mm* | - | Millimeter |
| *mg/g* | - | Milligram per gram |
| *V* | - | Volume |
| *w/v* | - | Weight per volume |
| *µg/mL* | - | Microgram per milliliter |
| *ABTS* | - | 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid |
| *BHT* | - | Butylated hydroxytoluene |
| *CSIRO* | - | Commonwealth Scientific and Industrial Research Organization of Australia |
| *DNA* | - | Deoxyribonucleic acid |
| *DPPH* | - | 2,2-diphenyl-1-picryl-hydrazyl-hydrate |
| *ET* | - | Electron transfer |
| *EPPO* | - | European and Mediterranean Plant Protection Organization |
| *FRAP* | - | Ferric reducing antioxidant power |
| *GAE* | - | Gallic acid equivalent |
| *GAS* | - | Group A *Streptococcus* |
| *GISD* | - | Global Invasive Species Database |
| *HAT* | - | Hydrogen atom transfer |
| *IAAPs* | - | Invasive alien aquatic plants |
| *INT* | - | p-iodonitrotetrazolium chloride |
| *MBC* | - | Minimum bactericidal concentration |

| | | |
|---|---|---|
| *MHA* | - | Mueller-Hinton agar |
| *MHB* | - | Mueller-Hinton broth |
| *MIC* | - | Minimum inhibition concentration |
| *QE* | - | Quercetin equivalent |
| *ROS* | - | Reactive oxygen species |
| *RPF* | - | Radical protection factor |
| *RSF* | - | Radical skin factor |
| *TAC* | - | Total alkaloid content |
| *TAE* | - | Tannic acid equivalent |
| *TE* | - | Trolox equivalent |
| *TFC* | - | Total flavonoid content |
| *TPC* | - | Total phenolic content |
| *TSC* | - | Total saponin content |
| *TTC* | - | Total tannin content |
| *UTHM* | - | Universiti Tun Hussein Onn Malaysia |

# LIST OF APPENDICES

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the study

Plants have been exploited as medicinal sources since ancient times. The World Health Organization (WHO) in 1993 stated that medicinal plants are not only important in the health care of ancient cultures for thousands of years due to their therapeutic potential but also in the current modern culture. Most consumers rely on herbal medications because alternative treatment options are more expensive and are frequently accompanied by dangerous negative effects (Mukheriee & Wahile, 2006). Accordingly, the use of natural plant products has been in greater demand nowadays and, as a result, there is a constant and urgent need to create new pharmaceuticals with a variety of chemical structures as well as brand-new mechanisms of action for infectious diseases that are currently emerging (Barbosa, Rall, & Fernandes, 2009).

Invasive alien aquatic plants (IAAPs) are non-native aquatic plant species that grow faster than native species, even in low-resource habitats (Jo, Fridley, & Frank, 2017). These plants are distinguished by their high level of adaptation in the absence of common predators, rapid proliferation, and phenotypic plasticity (Ismail *et al*., 2019). Besides, other variables that contribute to the establishment and spread of IAAPs include increased nutrient levels in water and changes in flow regimes (Keller, Masoodi, & Shackleton, 2018). They have a variety of effects on the socioeconomic system, freshwater ecology, and water quality (Hassan & Nawchoo, 2020). The IAAPs may obstruct fishing activity and water transportation by growing dense mats on the surface, blocking streams, limiting sunlight, and outcompeting native floating plants for nutrients. In addition, the occurrence of IAAPS in a habitat is recognized as the second largest threat to biodiversity after habitat loss (Ismail *et al*., 2019).

The rising of global trade and cross-border activity has enabled species to be transported to many parts of the world, including Malaysia and Indonesia. Both countries provide areas in which living organisms grow and prosper in humid and warm tropical regions. Some species colonize and naturalize well in their surroundings, while others become invasive (Ismail *et al*., 2019). In Malaysia, some of the aquatic plants discovered are on the list of the world's 100 worst invasive alien species (Jo *et al*., 2017), including water hyacinth (*Eichhornia crassipes*) and water fern (*Salvinia molesta*), and a few more that have yet to be named. As such, the introduction of these species would cause significant dangers to native species because they would face intense competition for resources, space, disease, and other factors (Ismail *et al*., 2019).

Despite being a nuisance to the ecosystem, this aquatic weed has enormous untapped medicinal potential that could make them effective therapeutic agent for a variety of developing diseases. Moreover, there is a growing global interest to discover the medicinal benefits of aquatic weeds (Nithya, Jayanthi, & Raghunathan, 2016). Reactive oxygen species (ROS) such as hydroxyl radicals, peroxides, and superoxide anions are primary causes of oxidative damage that leads to mutagenesis, aging, carcinogenesis, and cardiovascular diseases (Hajam *et al*., 2022). Both endogenous or exogenous antioxidants, whether synthetic or natural, protect the human body from oxidative stress-related disorders by inhibiting or delaying the oxidation process by limiting the onset or propagation of oxidizing chain reactions (Pisoschi *et al*., 2021). Currently, plant-derived natural antioxidants are in high demand due to their numerous health benefits (Manzoor *et al*., 2023).

Besides, since the introduction of antibacterial agents, they have played a critical role in reducing morbidity from infectious diseases. Nevertheless, infectious diseases continue to be the largest cause of death worldwide, and bacteria have become more resistant to conventional antibiotics in the recent years. The extensive usage of these compounds is expected to hasten the development of antibiotic resistance. In 2014, O'Neill published a high-profile assessment report estimating that antimicrobial resistance (AMR) might kill 10 million people per year by 2050. Accordingly, studies of natural products, with a focus on the isolation, purification, and characterization of secondary metabolites of plants, have has a significant impact on the discovery of substances with antibacterial action, because these metabolites have shown relevant benefits in the treatment of a variety of diseases (Bezerra *et al*., 2019; Mulat, Pandita, & Khan, 2019).

*S. molesta* D.S. Mitch is a worldwide aquatic invader, named after Antonio Maria Salvini (1633 – 1729), belonging to the family Salviniaceae. The species name, *molesta*, was derived from the Latin word *molestus*, highlighting its weediness as it is a nuisance across the world, invading natural water bodies as well as artificial impoundments (Julien, Hill, & Tipping, 2009). In Malaysia, this aquatic plant is also known as kiambang *Salvinia* (Baehaki, Herpandi, & Anggraini, 2018; Sidek *et al*., 2018; Maili, 2017). Based on the Global Invasive Species Database (GISD, 2022), in 2013, *S. molesta* was selected to replace the Rinderpest virus in the world's worst invasive alien species list, as the virus was declared eradicated in the wild in 2010.

Hence, in this study, *S. molesta* extracts, aqueous and ethanol, were examined to quantify the presence of phytochemicals and analyze the antioxidant and antibacterial activities. Understanding and evaluating the positive qualities of *S. molesta* extracts may be the initial step toward the effective exploitation of this plant as a natural alternative strategy for addressing IAAPs challenges in Malaysia. The knowledge of plant chemical contents is desirable for the identification of therapeutic agents as well as the discovery of new sources of economic phytocompounds for the synthesis of complex chemical substances and the actual significance of folklore medicines (Purushoth *et al*., 2013).

## 1.2 Problem statement

Taman Botani Johor is a public recreation area where both locals and visitors can relax and stroll around the lakes. *S. molesta*, however, has successfully infested the lakes in recent years and the management was negatively impacted by the thick mats of this invasive aquatic plant. *S. molesta* was manually harvested from the lakes because it was a sustainable practice. Even so, since not all plants are removed, thus allowing the population to be regenerated through vegetative reproduction (Hill & Coetzee, 2017), the harvest requires manpower and regular follow-ups. The biomass of *S. molesta* was left on the edges of the lakes after harvesting. As a result, the aesthetic value of the lakes eventually becomes tarnished, and the biomass is wasted.

According to Alves *et al*. (2021), one of the explanations for a successful, exotic invasive plant species is a 'novel phytochemistry hypothesis'—exotic plants possessing potent secondary compounds that are unique or underrepresented in the plants' new range, making it more likely to develop into highly invasive. In addition, plant materials for phytochemical studies are often expensive, scarce, and difficult to obtain. As suggested by Fan & Marston (2009), instead of focusing on rare or extinct species, research on the phytochemicals of common invasive species will utilize biomass as a source of potentially essential phytochemical compounds.

However, research and documentation on the phytochemicals and therapeutic values of *S. molesta* remain sparse and insufficient in Malaysia. Studies have focused more on the phytoremediation of this species on wastewater, as reported by Hayder & Mustafa (2021), Mustafa & Hayder (2021), Mohd-Nizam *et al*. (2020), Hanafiah, Mohamad, and Aziz (2018), Sidek *et al*. (2018), Ng, Samsudin, and Chan (2017), Ng & Chan (2017), and Ashraf, Maah, and Yusoff (2013). With the increasing interest in natural products, which may have less toxic and carcinogenic side effects than synthetic ones, researchers seek to assess and identify natural compounds as a green alternative (Johnston *et al*., 2005). Thus, the study aims to investigate the active compounds and medicinal values of the different extracts of *S. molesta*, which are aqueous and ethanol.

The production of highly ROS by a single unpaired electron causes oxidative stress and is implicated in the pathophysiology of a variety of physiological diseases. These include cellular injury, cancer, cardiovascular, aging, and other problems (Losada-Barreiro & Bravo-Diaz, 2017). Natural antioxidants derived from plant sources have gained significant public attention in recent years due to the negative side effects of synthetic antioxidants such as toxicity and carcinogenicity (Taghvaei & Jafari, 2015). Besides, George & Gabriel (2016) also asserted that plants are possible sources of natural antioxidants that can be used to sabotage ROS. Thus, a study that searches into the antioxidant potential of *S. molesta* is crucial for understanding the ability of the plant to operate as a natural antioxidant.

Microorganisms are the primary reason of acute and chronic diseases, and the regular use of antibiotics increases the number of novel strains with antibiotic resistance. Antibiotic resistance and the need for new antibiotics have emerged as a major concern due to the rising demand (Zada *et al*., 2021). As a result, the use of plant extracts is increasing. This is because plants contain a high concentration of antibacterial compounds and are being researched as a novel medication for antibiotic-resistant pathogens (Al-Rifai *et al*., 2017). Hence, the evaluation of *S. molesta* against the resistant bacteria is necessary to better understand the ability of the plant to combat the bacteria.

### 1.3 Significance of the study

Although aquatic weeds are an environmental annoyance, they also possess a significant untapped medicinal potential that could make them an effective treatment for a variety of emerging diseases (Nithya *et al*., 2016). Given the growing global interest in discovering the untapped medicinal properties of aquatic weeds, *S. molesta* might also be developed into a new, economically viable plant for medicine (Nithya *et al*., 2016). Even though there is no citation on the traditional use of *S. molesta* in prior literature, this invasive aquatic plant was reported to have potential antioxidant (Nithya *et al*., 2016) and antibacterial activities (Baehaki *et al*., 2018; Nithya, Jayanthi, & Raghunathan, 2015). The findings from this study provide essential opportunities for discovery, which may result in new insights and a valuable option for future demands, especially in medicinal values of an invasive aquatic plant, *S. molesta*.

### 1.4 Limitations of study

There are few limitations observed during this study. Firstly, the samples of *S. molesta* were collected from the lake of Taman Botani Johor, which was not a controlled environment. The findings in this study could be affected by the external environmental factors. Generally, it is recognized that variety of external factors, including biotic and abiotic stressors, light, air, and substrate composition, can have an impact on the growth, development, uptake of micro- and macronutrients, and accumulation of phytochemicals of the plants (Syakilla *et al*., 2022; Almuhayawi *et al*., 2021). Apart from that, only secondary metabolites of aqueous and ethanol extracts of *S. molesta* were investigated in this study. Thus, a solid conclusion on percentage yield could not be derived from the findings. This is because the primary metabolites were not measured. According to Do *et al*. (2014), high-polarity solvents can extract compounds with a wider polarity, which allows primary metabolites, such as proteins and carbohydrates, to dissolves during the extraction process.

## 1.5 Research objectives

The study aimed to investigate scientifically the potential medicinal values of an invasive aquatic plant, *S. molesta*. This aim could be achieved through following specific objectives:

i. To determine the phenolic, flavonoid, alkaloid, tannin, and saponin contents of aqueous and ethanol extracts of *S. molesta*.
ii. To study the antioxidant properties of aqueous and ethanol extracts of *S. molesta* using DPPH, ABTS, and FRAP assays.
iii. To examine the antibacterial properties of aqueous and ethanol extracts of *S. molesta* using the disc-diffusion method, minimum inhibition concentration (MIC), and minimum bactericidal concentration (MBC) assays.

## 1.6 Scope of the study

The scopes of the study are as follows:

i. Taman Botani Johor, Batu Pahat was chosen as the study site for the collection of *S. molesta* samples.
ii. *S. molesta* samples were extracted using aqueous and ethanol solvents.
iii. The extracts were analyzed on phytochemical content including phenolics, flavonoids, tannins, alkaloids, and saponins.
iv. The antioxidant activity of both extracts was measured using free radical scavenging assays.
v. Four selected bacteria were used for the antibacterial activity test, specifically two Gram-negative: *Escherichia coli* and *Pseudomonas aeruginosa*, and two Gram-positive: *Bacillus cereus* and *Staphylococcus aureus*. Different bacteria were chosen to show the ability of the extracts as an antibacterial agent, using the disc diffusion method, MIC, and MBC assays.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Aquatic plants

Aquatic plants, also called hydrophytic plants or hydrophytes, are plants that have adapted to or are living in aquatic environments (Muda, 2010). The three main divisions of aquatic plants include bryophytes (mosses, liverworts, and hornworts), pteridophytes (ferns and allies), and spermatophytes (seed-bearing plants) (Table 2.1) (Bornette & Puijalon, 2009; Chambers *et al.*, 2007). These aquatic plants are also classified into emergent, floating leaf, and submerged growth forms (Ismail, 2014; Chambers *et al.*, 2007).

Table 2.1: Aquatic plant divisions and representative genera (Chambers *et al.*, 2007)

| Kingdom | Division | Descriptive term | Representative genera |
|---|---|---|---|
| Plantae | Bryophyta | Mosses and liverworts | *Fontinalis*, *Riella*, *Ricciocarpus* |
| | Pteridophyta | Ferns and allies | *Azolla*, *Salvinia*, *Isoetes* |
| | Spermatophyta | Seed-bearing plants | *Sagittaria*, *Alisma*, *Butomus*, *Brasenia*, *Cabomba*, *Callitriche*, *Ceratophyllum*, *Scirpus*, *Carex*, *Myriophyllum*, *Ellodea*, *Vallisneria*, *Juncus*, *Lemna*, *Utricularia*, *Nelumbo*, *Nymphaea*, *Nuphur*, *Sapartina*, *Eichhornia*, *Potamogeton*, *Ranunculus*, *Sparganium*, *Typha* |

In the early 21st century, researchers have come to understand the critical role of plants that grow in or near water play in the development, maintenance, and provision of aquatic ecosystem services (Chambers *et al.*, 2007). In addition to interacting with and influencing the hydrological, geomorphological, and physiochemical environments, aquatic plants also engage in a wide range of interactions with other organisms from microbes to vertebrates, for example, by providing habitat and food (O'Hare *et al*., 2018; Wood *et al*., 2017). Aquatic plants are the foundation of aquatic food chains; they also actively contribute to the development and maintenance of freshwater ecosystem services and food webs (Smith, 2011; Scheffer & Jeppesen, 2007). These plants provide ideal habitat for large fish, besides serving as a shelter for the juvenile and food sources for fish, insects, waterfowls, and other wildlife.

All plants, including those that grow underwater, produce oxygen due to photosynthesis; thus, aquatic plants are also the primary source of oxygen for other aquatic life (Bonvechio & Bonvenchio, 2006). Moreover, aquatic plants act as important bioindicators of environmental conditions and long-term ecological changes in water quality (Lacoul & Freedman, 2006; Solimini, Cardoso, & Heiskanen, 2006). Rooted aquatic plants help to stabilize shorelines and bottom sediments. They absorb nutrients and filter pollutants from runoff, resulting in cleaner water. Lembi (2009) added that a diverse aquatic plant population enhances the beauty of the water body. Thus, many people acknowledge and appreciate the aesthetic value of aquatic plant life, whether in a backyard fishpond, around a retention pond, or along the shoreline of a lake.

### 2.1.1 Invasive alien aquatic plants (IAAPs)

The introduction of non-native aquatic plants to the new habitat has frequently become a nuisance by impeding human water use and endangering the structure and function of diverse native aquatic ecosystems (Richardson *et al*., 2000). Invasive alien aquatic plants (IAAPs) are defined as non-native aquatic plant species that grow more quickly than native species, even in habitats with limited resources. The rapid growth of invasive plants is associated with a set of plant traits that can boost nutrient uptake and photosynthetic capacity (Jo *et al*., 2017). According to Gettys (2014), some of the IAAPs are purposely introduced by humans as ornamentals, aquarium decorations, and souvenirs. Some introduced aquatic plant species are colonized and naturalized well to the native environment, while some become invasive. Besides, the invasion of these species in the native habitats was also caused by the expanding global trade and cross-boundary transportation services (Havel *et al*., 2015).

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# VITA

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