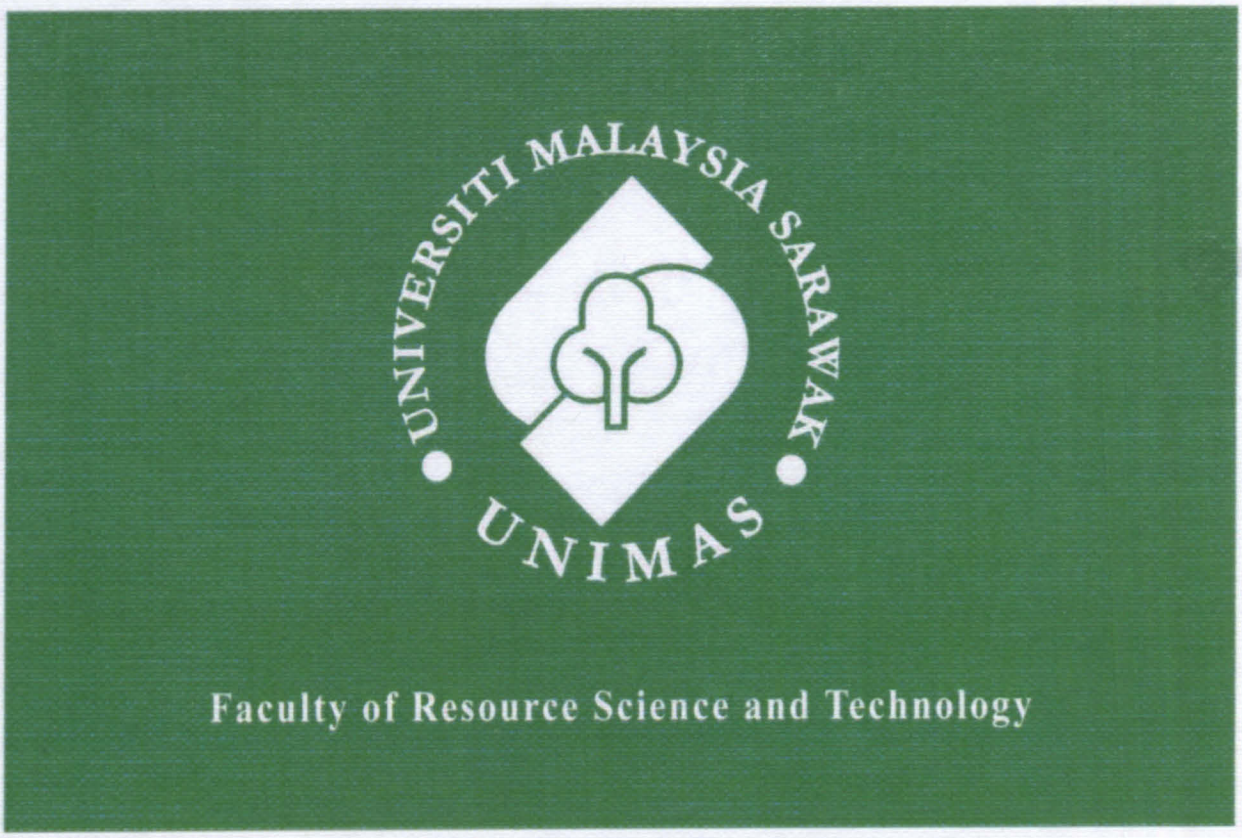

Screening of Medicinal Plants against *Aspergillus flavus*

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53638

Bachelor of Science with Honours
(Resource Biotechnology)
2018

Borang PTA4

Borang Pengesahan
Laporan Projek Tahun Akhir (STF3015)

Fakulti Sains dan Teknologi Sumber
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Masters ☐

PhD ☐

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Screening of Medicinal Plants against *Aspergillus flavus*

Ravi Kumar a/l Kalaichelvam

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A progress report submitted in partial fulfillment of the

Final Year Project 2 (STF3013) course

Supervisor: Ms. Rosmawati Bt Saat

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Declaration

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Ravi Kumar Kalaichelvam (53638)

Resource Biotechnology

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Screening of Medicinal Plants against *Aspergillus flavus*

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Abstract

This project focused on antifungal activity of *Azadirachta indica, Ficus religiosa* and *Mentha piperita*_leaves extract in order to evaluate the effect of methanol extracts against the most important phytopathogenic fungi, *Aspergillus flavus*_by poisoned food technique and disc diffusion technique. Different concentrations which were 100, 75 and 50 mg/ml of these extracts were found to inhibit the growth *A.flavus*. In the solvent extracts tested, *Mentha piperita* extract gave the highest percentage of inhibition which was 31.25% against *Aspergillus flavus*. The different concentration of methanol extracts has showen inconsistent percentage of growth of inhibition for *Aspergillus flavus* which it was slightly not effective in antifungal activity of *Azadirachta indica*, and *Ficus religiosa.*

Keywords: *Azadirachta indica, Ficus religiosa, Mentha piperita,* phytopathogenic, poisoned food technique, disc diffusion technique

Abstrak

Projek ini memberi tumpuan kepada aktiviti antikulat daun Azadirachta indica, Ficus religiosa *dan* Mentha piperita *ekstrak untuk menilai kesan ekstrak metanol terhadap kulat,* Aspergillus flavus *oleh teknik makanan beracun dan teknik difusi cakera. Kepekatan yang berbeza iaitu 100, 75 dan 50 mg/ ml ekstrak ini didapati menghalang pertumbuhan* Aspergillus flavus. *Dalam ekstrak pelarut yang diuji, ekstrak* Mentha piperita *memberikan perencatan peratusan tertinggi iaitu 31.25% terhadap* Aspergillus flavus. *Kepekatan yang berbeza dari ekstrak metanol menunjukkan peratusan pertumbuhan yang tidak konsisten untuk* Aspergillus flavus *yang sedikit tidak berkesan dalam aktiviti antikulat* Azadirachta indica, *dan* Ficus religiosa.

Kata kunci: Azadirachta indica, Ficus religiosa, Mentha piperita, *teknik makanan beracun, teknik difusi cakera*

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List of Abbreviations

DMSO	dimethyl sulfoxide
A. flavus	*Aspergillus flavus*
PDA plate	Potato dextrose agar plate
A. indica	*Azadirachta indica* (neem)
F. religiosa	*Ficus religiosa* (peepal)
M. piperita	*Mentha piperita* (mint)
%	Percentage
mg/ml	milligram per mililitre
g	Gram
cm	centimeter

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1.0 Introduction

Variety types of plants are in use by conventional, medicinal and traditional medicine to improve and endure health and to treat minor illnesses (Anderson *et al.*,2000).To be more specifically, some of these plants are frequently used to lower the cholesterol as anti-inflammatory, antioxidant, antifungal and neuro protective against cerebral insufficiency in the elderly or as chemo-preventive agents. Apart from that, some of these products are also consumed to boost the immune system of human being (Newman, Yang, Pawlus, & Block, 2008).

Plants are considered as a potential source of medicinal compounds. Traditionally, plants are used in oral health and to treat many diseases such as infectious diseases, diarrhoea, fever and cold (Odenholt, Lowdin, & Cars, 2001). According to World Health Organization (WHO) definition, a medicinal plant is a plant that can be used for therapeutic purposes and or its compound can be used as a pioneer in the synthesis of semi-synthetic chemical drugs (World Health organization [WHO], 1979). Around 250,000 plant species are in the world, but only 6% have been screened for biological activity. According to Farnsworth *et al.* (1985), there are 119 plant-derived drugs used today all over the world, and all of those came from less than 90 plant species. The possibility of finding novel compounds from plants that can be exploited for medicinal use is enormous.

There is increasing in number of fungal resistant to various antifungal. Due to that, several attempts to use the antifungal potential of plants have been carried out. Antifungal compounds that found in plants with different mechanisms of action against resistant microbial strains are of clinical importance (Mares, 2004). Based on the importance of medicinal plants in Pakistan it is estimated that an 80% of its population depend on plant to cure themselves, a 40% in China. In developing and advanced countries as the United States,

it is estimated that 60% of its population use medicinal plants normally to fight certain diseases.

The medicinal plants are applied in pharmaceutical, cosmetic, agriculture and food industry. Proves are documented in history of all civilizations where medicinal plants are used for curing the diseases. During that era, peoples were probably not aware about the health hazards associated with irrational therapy. With start of research in medicine, it was determined that plants contain active principles, which are responsible, for curative action of the herbs (Jeff-Agboola & Onifade, 2016). Medicinal and aromatic plants are economically important plants where they provide basic raw materials for medicines, perfumes, flavours and cosmetics. The plants and their products serve as valuable source of income for small holders and entrepreneurs and also help the country to earn valuable foreign exchange by way of export (Sultabhaha, Suttajit, & Niyomca, 1992).

Aflatoxin is a toxin that produced by the fungi such as *Aspergillus flavus*. Aflatoxin can contaminate a wide variety of food and feed commodities including maize, oil seeds, spices, groundnuts, tree nuts, milk and dried fruits. Quality and quantity of food and feed materials is decreased due to the presence of aflatoxins in food chain. In addition, consumption of aflatoxin-contaminated products can cause a risk of development of various diseases in human and animals. The crops can attack by *Aspergillus* at any different times, in the field, during harvest, transport and storage. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market.

Therefore, the objectives of this study are;

1. to analyse and determine the effectiveness of four medicinal plants which is *Azadirachta indica* (neem), *Ficus religiosa* (peepal) and *Mentha piperita* (mint) leaves against toxigenic *A. flavus.*

2. to evaluate the minimum inhibitory concentration of the plants extracts against the toxigenic *A. flavus.*

3. to investigate the phytochemical properties of the plant extracts.

2.0 Literature review

2.1 *Aspergillus flavus*

Previously, fungi are considered as plant kingdom but due to lack of chlorophyll and unique structural and physiological feature, fungi are separated from the plant kingdom (Hua, 2013). Most of the fungi are free living in soil or water while others form a parasitic or symbiotic relationship with plants or animals. There is 99,000 known species of the kingdom fungi (Zain, 2011). Yeasts, rusts, smuts, mildews, mold, and mushrooms are included in the kingdom fungi. Fungi are different compared to other living organisms including animal due to the principal mode of vegetative growth and nutrient intake. Commonly fungi consist of branched mass, tubular filaments covered by rigid cell walls. Fungus is eukaryotic where they contain membrane-bound organelles and clearly defined nuclei (Campbell, 1994). Filaments called hyphae, the branches are repeatedly a complex and widespread network known as mycelium, which forms thallus, or undifferentiated body, from ordinary mushrooms (Campbell, 1994). The hyphae's wall is complex in both composition and structure. Fungal spores germinate and form hyphae under favourable environmental conditions. The cytoplasm will be activated when the spores absorbs water thru the wall. Because of this, the nuclear division will take place. Carbohydrates and proteins can be the carbon source for the fungi (Campbell, 1994).

A. flavus is a type of fungus that are commonly exists in the soil. However, without a clear reason, this type of fungus can also be found in the air of some hospitals and locales (Hedayati *et al.*, 2007). It is an opportunistic pathogen to the crops such as peanut, cotton seed and maize, where it survived on organic nutrients source such as tree leaves and decaying woods (Klich, 2007).

A. flavus is classified under Ascomycota filamentous fungi (Pandey *et al.*, 2000). It grows by producing sclerotia that will germinate to produce either conidia or hyphae (Hedayati *et al.*, 2007). Hyphae have a lot of networks that are known as mycelium which secretes the enzymes that can break down food sources into small molecules. The mycelium will then absorb the molecules that will provide fuel to the fungus which will encourage the growth of *A. flavus* (Scheidegger & Payne, 2003). The hypal mode of proliferation of fungi gives a large advantage of *A. flavus* compared to unicellular microorganism in taking over the solid substrates and also aid in maximum utilisation of nutrients available in the substrate (Mienda *et al.*, 2011).

Naturally, *A. flavus* is a saprophytic fungus which is able to grow on various organic sources such as tree leaves, indoor and outdoor air environment, dead animals, sick humans and plant in the soil. Hence, this fungus is very crucial in nutrient cycling (Hedayati *et al.*, 2007). However, the growth of *A. flavus* on food source may leads to aflatoxin contamination (Campbell, 1994). This is because, *A. flavus* absorb nutrients from the host plants through hydrolytic activity where it utilises enzymes such as amylase, pectinase and cellulases. These hydrolytic enzymes may contribute to pathogenicity of *A. flavus* (Jiujiang *et al.*, 2005).

The infections of *Aspergillus flavus* in maize and peanut and subsequent contamination with aflatoxin cause a threat to global food safety and human health, and is worsen by drought stress (Fountain, 2014). Drought stress-responding compounds such as reactive oxygen species (ROS) are associated with fungal stress responsive signaling and secondary metabolite production, and can stimulate the production of aflatoxin by *A. flavus in vitro* (Fountain, 2014).

2.2 Medicinal Plants

Medicinal plants now have an important interpretation because of their special properties as a therapeutic phytochemical source that can lead to the development of new drugs. A rich source is obtained from the plants for the discovery of new drugs. A number of bioactive compounds can be isolated from different parts of a single plant. Most of the modern research stated that the majority of ethnobotanical claims are valid and relate with our current knowledge of plant-derived compounds. Through scientific research, benefits of traditional plants have been explored, which led to the discovery of many valuable drugs for the modern world. Examples include reserpine from *Rauwolfia serpentina* (Indian snake root), vincristine from *Catharanthus roseus* (Madagascar periwinkle), artimisinin from *Artemisia annua*, (sweet sagewort), capsaicin from *Capsicum annuum* (chili pepper), morphine from *Papaver somniferum* (poppy), atropine from *Atropa belladonna* (deadly night shade), silymarin from *Silybum marianum* (milk thistle), and ephedrine from *Ephedra sinica* (Chinese ephedra) (Gupta, et al., 2005).

Most of the phytochemicals from plant sources such as phenolic and flavonoids have been reported to have positive impact on health and cancer prevention (Venugopal, 2012). The high content of phenolic and flavonoids in medicinal plants are preventing the development of age-related disease. The researches on medicinal plants are getting high because of the beneficial phytochemicals in medicinal plants and development of natural products in pharmaceuticals and cosmeceutical industry. Several studies have been performed examining the effects of antioxidants on the growth and aflatoxin production of *Aspergilli*. For example, phenolic compounds such as caffeic acid tannic acid derived from tree nuts have been shown to inhibit aflatoxin production in *A. flavus* (Mahoney *et al.*, 2010).

2.2.1 Azadirachta indica (neem) leaf

Azadirachta indica (*A.indica*) plant is originally from Southeast Asia, this plant is also found in tropical and semitropical regions like India, Bangladesh, Pakistan and Nepal. *A. indica* tree belongs to the family Meliaceae (Alzohairy, 2016) and its most important active constituent is azadirachtin (Hossain et al., 2011). The medicinal properties of the Neem leaf attract worldwide. Neem has been widely used in Ayurvedic, Unani and Homoeopathic medicines and has become the focus of modern medicine. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, anti-hyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antioxidant, anti-mutagenic and anti-carcinogenic properties (Subapriya, 2005). Neem contains a number of active biological compounds chemically different and structurally variable with more than 140 isolated compounds from various parts of the tree (Subapriya, 2005). Quercetin and B-sitosterol was the first polyphenolic flavonoids purified compound from neem fresh leaves and they were having antibacterial and antifungal properties. Neem leaf is using for treat few skin infections such as eczema and psoriasis. Short term usage of neem is safe while long term usage of neem may harm to kidney or liver. Neem also can cause miscarriages, temporary infertility and low blood sugar (Subapriya, 2005).

Neem is a rich source of limonoids that are capable with potent medicinal properties predominantly antioxidant, anti-inflammatory, and anticancer activities and Azadirachtin, gedunin, and nimbolide are more extensively investigated relative to other neem limonoids (Nagini, 2014). The chemical components which may contain many biologically energetic compounds that can be extracted from neem like alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Azadirachtin is an amalgamation of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more efficient. The chemical constituents in crude extracts of the leaves of *Azadirachta indica* (neem) is

identified by using modern sensitive gas chromatography–mass spectrometry (GC–MS) (Hossain, 2011). Majority of the identified compounds in crude extracts contain normal hydrocarbons, phenolic compounds, terpeniods, alkaloids and glycosides and the high percentage of compounds that were identified in the crude extracts are chemically and biologically important (Hosaain, 2011).

Table 1: Phytochemical screening of crude extracts of neem (Dash, 2017)

Compound	**Presence or Absent (Methanolic Extract)**
Alkaloids	Presence
Glycosides	Presence
Saponins	Absent
Flavanoids	Presence
Reducing sugar	Presence
Tanins	Presence

2.2.2 *Ficus religiosa* (peepal) leaf

Ficus religiosa tree is a large perennial tree, largely planted as an avenue and roadside tree especially near temples. Peepal has good medicinal value as well. It is widely used in Ayurveda. Each part of the tree can be eaten and it offers a lot of benefits. The morphology of the leaf is are shiny, thin, and bear 5 to7 veins. Peepal leaf is used in the treatment of various infections, healing wounds, enhancing fertility and treating poisoning. The high contain of phenolic and flavonoids compound in the leaf, it also effective for antimicrobial and antifungal. Antibacterial effect against *Staphylococcus aureus, Salmonella paratyphi, Shigella dysenteriae, S. typhimurium, Pseudomonas aeruginosa, Bacillus subtillis, S. aureus, Escherichia coli, S. typhi* are showed from the aqueous and ethanolic extracts of *F. religiosa*

leaves (Valsaraj, 1997). According to Jung (2008), the methanolic extract of *F. religiosa* leaf inhibits the production of nitric oxide and proinflammatory cytokines in lipopolysaccharide (LPS) stimulated microglia via the mitogen activation protein kinase (MAPK) pathway by using cell viability assay, nitric oxide assay, and enzyme-linked immunosorbent assay (ELISA). Apart from that, the leaves are purgative and tonic. They are useful in constipation and jaundice. Their oral intake strengthens the liver and helps control the complaints. They are also given to treat the condition of the fever and to stop the bleeding or seepage.

The methanolic extract of *F. religiosa* leaf inhibits the production of nitric oxide and proinflammatory cytokines in lipopolysaccharide (LPS) stimulated microglia via the mitogen activation protein kinase (MAPK) pathway (Jung, 2008). The extract applies strong anti-inflammatory properties in microglial activation. Peepal leaves contain campestrol, stigmasterol, isofucosterol, α-amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tryosine, methionine, valine, isoleucine, leucine, n-nonacosane, n-hentricontanen, hexa-cosanol and n-octacosan (Behari, 1984).

Table 2: Phytochemical screening of crude extracts of Ficus religiosa (Singh, 2011).

Compound	**Presence or Absent (Methanolic Extract)**
Alkaloids	Absent
Carbohydrates	Presence
Saponins	Presence
Phenols	Presence
Flavanoids	Presence
Protein	Presence
Tanins	Presence
Terpenoids	Presence