# PRELIMINARY STUDIES OF ANTIFUNGAL AND PHYTOCHEMICAL PROPERTIES OF *Mimusops elengi* SEED EXTRACTS AGAINST PADDY GRAIN FUNGI

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## ABSTRACT

*The ever revolving fungi strains which are building resistance towards conventional fungicides requires continuous search for alternatives. Environmental and health concerns due to the current practice of synthetic pesticides usage in agricultural fields have encourages more venture into bio-pesticides research. Various plants extractives have been tested and proven to contain active compound asserting antifungal properties. One of them, Mimusops elengi, a widely available endogenous plant in tropical countries and most parts of this plant have been proven to possess antifungal effect towards various species of fungi. Antifungal properties of crude extractives from the seeds Mimusops elengi are tested on paddy grain fungi. Extraction of Mimusops elengi seeds were done using water, methanol, ethyl acetate, dichloromethane and petroleum ether as solvents and extractives were tested in vitro for antifungal activities on paddy grains. Water extract shows the most significant antifungal effect followed by methanol extract, dichloromethane extract, ethyl acetate and petroleum ether extract. Grains treated with the seeds extract and when compared to the surface disinfected grains during seven days observation shows that water has the highest fungi inhibition properties at 58% followed by methanol (54%), dichloromethane (40%), ethyl acetate (38%) and petroleum ether (32%). Antifungal ability of the seed extracts when compared to both synthetic fungicide, thiram and mancozeb at recommended dosage shows water and methanol extract asserts very close antifungal effect as the synthetic ones. Antifungal properties of a plant are largely linked to the plant's derivatives and active constituents. Qualitative phytochemical test of Mimusops elengi seeds extracts show positive presence of the compounds saponin, flavonoid, triterpeniods, tannins, phenol as well as cardiac glycosides which are plants active compounds frequently related in antimicrobial activities studies. This preliminary screening could narrow down the potential of this seed extracts as natural antifungal agents and the acting active compounds. Further exploration into this plant extracts as synthetic pesticides alternatives can benefits the management of paddy diseases especially towards pathogenic species with a greener approach.*

**Keywords:** Antifungal, fungicide, *Mimusops elengi*, paddy grain, phytochemicals.

## INTRODUCTION

Rice (*Oryzae sativa*) is the most essential crop and widely cultivated in Asian region. In Malaysia alone rice fields covers about 690,000 hectares and produces 1.8million tonnes of rice [1]. Rice like other cultivated plants faces the same challenges when comes to diseases and infection caused by pest, fungi, bacteria, viral and mycoplasma pathogens [2]. According to [3], more than 50 pathogenic seed borne fungi species from paddy have been reported and some of the most common species found in paddy across the globe includes Fusarium, Aspergillus, Cercospora, Pyricularia, Alternaria, Curvularia, Rhizoctonia and Trichoderma causing harvest infection, seed rot, seed necrosis, loss of germination capability, loss of grain nutrition value and seedling damage [4- 5].

Treatment directly on seeds are the most effective and economical approach in controlling fungal diseases from seed and maintaining quality of grains [6]. Synthetic pesticides which are largely used by paddy farmers in Malaysia includes mancozeb, maneb, mathalaxyl, thiram and azoxystrobin. Even though synthetic pesticides could efficiently control fungal growth on grains but they should not be applied on grains for they might cause pesticides toxicity directly to consumers and active continuous use of chemical fungicides could cause serious damage to the environment and other non-targeted organisms [7]. Farmers need to increase the concentration of fungicides used or change to a new and more prevalent fungicides over as all antimicrobials have a certain life span in which case microorganisms can develop resistance towards a certain drug. Hence, to overcome those issues, continuous search for new and safer antimicrobial drugs or biopesticides should be done to ensure an eco-friendly pest control practices.

Wide range of plants and their derivatives have been used for its medicinal, pharmaceutical and therapeutic benefits. Plant metabolites as fungicides could be an alternative practice compared to common synthetic fungicide used. Extracts from plants such as *Syzygium aromaticum, Emblica officinalis* Gaertn., *Allium sativum* L., *H. anthelminthicus*, *X. lancelatum* and *C. sappan*, *Azadirachta indica* (neem) and *Persea americana* (pear) reported to exhibit antifungal effect in laboratory studies against paddy and grain moulds [8-10]. *Mimusops elegi* plant is an evergreen tree usually cultivated as decorative or shade tree. Its seeds have been traditionally used as remedy for piles, headache, constipation and spermicidal [11]. Barks and leaves of this plant are also proven to be exhibit significant antimicrobial characteristics towards several species of bacteria and fungi [12]. Therefore, in this study, antimicrobial potential of this plant is further tested on its ability to inhibit fungal growth from paddy grains and phytochemicals of the plants are qualitatively screened to preliminarily ascertain active compounds from *Mimusops elengi* responsible in exhibiting antifungal characteristics.

## METHODOLOGY

### Collection of Paddy Grains Samples

Paddy grains samples are collected from farm fields during harvest season and rice mills $9^{th}$ to $12^{th}$ January of year 2015 and 2016 from region of Pulau Pinang and Kedah, Malaysia. Collected samples are bagged in sterile plastic container and kept in 4°C refrigeration.

### Preparation and Extraction of *Mimusops elengi* Seeds

Ripe fresh fruits of *Mimusops elengi* (Sapotaceae) were collected from its trees growing in Esplanade Bay, Pulau Pinang and seeds were separated from flesh. Seeds are washed thoroughly with sterile distilled water, dried in dryer at 40°C for a week, powdered and used for extraction. The powdered seeds are macerated separately using water, methanol, ethyl acetate, dichloromethane and petroleum ether as extraction medium at 1:20 seed to solvent ratio for three days. The extracts are concentrated of their solvent and preserved at 4°C in air tight dark bottle till further use.

### Antifungal Bioassay

In vitro antifungal activity assay were performed with water, methanol, ethyl acetate, dichloromethane and petroleum ether extract of *Mimusops elengi* seeds against fungal growth on paddy grains using seed surface treatment and direct plating method. The concentrated each extracts are dissolved into different vials to 2500ppm concentration using respective solvents for antifungal test. Conventional pesticide thiram and mancozeb were prepared in aqueous according to its recommended concentration 2000ppm and 2500ppm respectively. Fifty of the collected paddy grains were surface disinfected with 2% sodium hypochlorite solution for two minutes and rinse twice with distilled water to eliminate surface contamination during collection and transference before treated with the *Mimusops elengi* water extract. This step were then repeated with the methanol, ethyl acetate, dichloromethane and petroleum ether extract as well as thiram and mencozeb. The grains are allowed to dry at room temperature after ensuring all the grains surface have come in contact with the solutions before being plated onto potato dextrose agar medium amended with antibiotic streptomycin and neomycin at ten grains per plate. Plated samples were incubated at 30°C and the antifungal activity assay were determined by counting the number of non-disinfected grains over seven days observation.

### Phytochemical Screening of *Mimusops elengi* Seeds

Standard laboratory method are used for qualitative phytochemical screening on each of the *Mimusops elengi* seeds extracts. Phytochemical properties screened includes carbohydrate, reducing sugar, saponin, flavonoids, alkaloids, terpenes, tannins, phenol and glycosides. Presence carbohydrates and reducing sugar are tested using Molisch's test and Benedict's test respectively. Saponin are tested using Frothing test while flavonoids with Shinoda Test. Dragendorff's test are used to confirm presence of alkaloids while Liebermann-Burchard test for triterpenoids. Both tannins and phenol can be tested for presence using ferric chlorides test whereas Kellar-Kiliani test confirm presence of cardiac glycosides. These methods allow phytochemical properties contain in the extracts to be known qualitatively through observation of colour changes that happen to the extract solutions during tests [13].

## RESULTS

### Preliminary Treatment of Paddy Grains with *Mimusops elengi* Seeds Extracts

Figure 1 presents the trends of antifungal activity of the extracts and conventional pesticides on surface disinfected paddy grains (in percentage) for seven days observation. Non-surface disinfected paddy grains which are directly plated after collection shows 94% infected grains at day one of observation and full infection at day four. The high fungal growth may be caused by cross contamination during collection and transference hence the collected paddy grains are surface disinfected before treatment process. The surface disinfected grains shows only 6% of infected grains on day one however fungi grow exponentially reaching 98% infection on day seven. Among the five seed extracts treatments on the grains, water extract of shows the most significant antifungal effect followed by methanol extract, dichloromethane extract, ethyl acetate and petroleum ether extract. When comparing treated grains with surface disinfected grains as control, result of the highest percentage of fungal inhibition along seven observation days, water extracts inhibited grain infection by 58% followed by methanol (54%), dichloromethane (40%), ethyl acetate (38%) and petroleum ether (32%) while conventional pesticide thiram and mancozeb indicate 62% and 55% suppression, respectively. Water and methanol extract shows a very close if not similar trend in the grain fungal inhibition as conventional pesticides thiram.

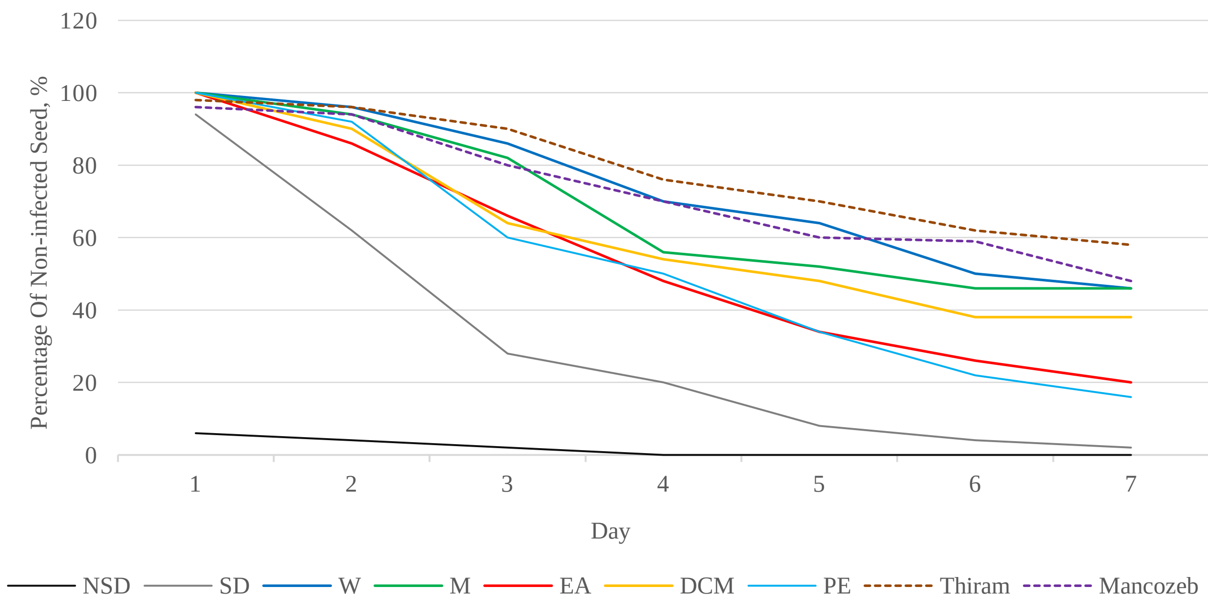


**Figure 1.** Percentage of Non-Infected Paddy Grains after Surface Treatment with Mimusops elengi Seeds Extracts and Conventional Pesticides Thiram and Mancozeb during Seven Days Observation.

NSD= Non-surface disinfected paddy grains sample; SD= Surface disinfected paddy grains sample,; W= Water extract treated paddy grains sample; M= Methanol extract treated paddy grains sample; EA= Ethyl acetate extract treated paddy grains sample; DCM= Dichloromethane extract treated paddy grains sample; PE= Petroleum ether extracts treated paddy grains sample; Thiram= Thiram solution treated paddy grains sample and Mancozeb= Mancozeb solution treated paddy grains sample.

**Phytochemical Properties of *Mimusops elengi* Seeds**

Each of the *Mimusops elengi* seeds extracts are subjected to qualitative phytochemical screening and results obtained are as in Table 1. Carbohydrates are detected in all solvents extracts but only water and methanol extracts contain reducing sugar. Saponin, flavonoids, alkaloids, tannins and phenol are found to be present in both water and methanol extracts. Triterpenoids are detected in all five types of solvents extracts while cardiac glycosides are only tested positive in ethyl acetate and dichloromethane extracts. Majority of the phytochemicals compound are found in both strongly polar solvent extracts namely water and methanol.

**Table 1:** Qualitative Phytochemical Screening of *Mimusops elengi* seeds Different Solvents Extracts.

| Phytoconstituents | Observation | | | | |
|---|---|---|---|---|---|
| | W | M | EA | DCM | PE |
| Carbohydrates | + | + | + | + | + |
| Reducing Sugar | + | + | - | - | - |
| Saponin | + | + | - | - | - |
| Flavonoids | + | + | - | - | - |
| Alkaloids | + | + | - | - | - |
| Triterpenoids | + | + | + | + | + |
| Tannins | + | + | - | - | - |
| Phenol | + | + | - | - | - |
| Cardiac Glycosides | - | - | + | + | - |

W= Water extract; M= Methanol extract; EA= Ethyl acetate extract; DCM= Dichloromethane extract; PE= Petroleum ether extracts.

## DISCUSSION

Research have been actively done in recent years in search for alternatives for synthetic pesticides. Studies by [16-19] have shown potential derivatives and extracts from various plant species as a promising alternative as antifungal agents for paddy diseases and plants should be further exploited for pest management studies as Hamburger and Hostettmann [20] proclaimed that there are more than 10, 000 secondary metabolites obtained from plants which plays important role in the plants natural defence system. More screening and evaluation on varieties of plant ought to be done to uncover the potential active compound and constituents which are economically viable new sources of pesticide. In this study, preliminary screening has revealed that the chemical constituents from *Mimusops elengi* seeds crude extracts possess active compounds which inhibit fungal activities on the grains. All five solvents extracts shows a certain degree of inhibition of fungal growth on paddy grains when compared to the surface disinfected paddy grains could signify the fungal inhibitory role which the extracts have. Additionally, similar solvents extracts from barks, leaves and fruits crude extracts has been tested to exhibit antimicrobial properties as exclaimed by Ali et. al [12]. This suggest that some derivatives from *Mimusops elengi* plant are able to act as fungal growth suppressant.

Qualitative phytochemical screening provides an understanding of possible active constituents which are fungi inhibitory. Having most of the phytochemicals compounds present in the water and methanol extracts may explains the performance of both extracts having high fungal inhibitory effect which are almost similar to antifungal effect of tested synthetic fungicide. For example phytochemical saponin, a complex sugar distributed in plants and are associated to triterpene or steroid through a hydroxyl group or a carboxyl group well known for surfactant properties [21] and have been known for its medicinal new sweetener and flavor enhancer, exhibited inhibitory effects against fungi and yeast [22- 23]. Phytochemical such as flavonoid, alkaloids, tannins, triterpenoids and phenols are phytochemicals which have been actively investigated and proven to a certain extend in recent years for their cytotoxicity, medicinal, antibacterial and antifungal properties. Published work [24-29] have all shown derivatives of these phytochemicals plays crucial roles in the bioacitivities of the compound towards pathogens. Further separation and identification work on the antifungal crude extracts could help to derive useful chemical compounds against pathogenic fungi.

Plant based fungicides could be a better alternative for their greener approach towards non-target organism and environmental health. Findings from current study prepares an essential direction about isolation and identification of antifungal consituents that can be further instigated into rice protection along its production line. With the results obtained, *Mimusops elengi* plant crude extracts which can be a great candidate against pathogenic fungi that causes rice diseases. However, before direct application of the crude extracts as fungicide, further investigation needed to be done to identify the responsible acting compounds and substantiating its effectiveness.

## CONCLUSION

*Mimusops elengi* is an invaluable plant source which have been used traditionally for its drug effect. Both water and methanol crude extracts of the plant have shown promising antifungal properties which are up to par as the synthetic fungicides compared in this study. This may be due to the broad range of phytochemical properties it possesses and are playing an active role in fungal growth inhibition. An extended studies could help isolate and identify active compounds which can serve not only as a more environmental friendly choice of microbial treatment but may also useful drugs in broad range of industrial applications.

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