Anti-Fungal Effect of Chloroform Crude Extract from the Leaves of *Thottea Corymbosa*

Siti Zaiton Mat So'ad
Neni Kartini Che Mohd Ramli
Aiza Harun

ABSTRACT

Bioactivities of the crude extracts from the leaves of Thottea corymbosa were tested on Brine Shrimp Lethality and anti-fungal activity. Three genus of fungal were tested for anti-fungul activity i.e. Rhizoctonia solani, Sclerotium rofsii and Pythium sp., the pathogens of vegetables. The repeated test was done by using other fungi i.e. Aspergillus niger, Aspergillus flavus and Penicillium sp. from the building molds. The in-vitro anti-fungal activities of Thottea corymbosa extracts against microorganism revealed that there was no inhibition zone occured for the three genus of fungal tested. This revealed that there were no anti-fungal activities of crude extract against plant pathogens. The repeated test against the building molds revealed that only chloroform extract had a little anti-fungal activities against Penicillium sp.

Keywords: *Thottea corymbosa, building molds, plant pathogens, anti-fungal activities*

Introduction

Nowadays, the discovery of new antimicrobial agents for human uses and veterinary therapeutic has become an urgent need since current drugs increase in severity and extent. For instance, according to a survey, it had been discovered that the parasite from *Plasmodium falciparum* strains, which is responsible for many fatalities from malaria has become a resistant to chloroquine. This phenomenon has also occurred with some important pathogenic bacteria and, in that case, billions of people worldwide are affected and suffered by this bacterial disease. Hence,

the identification of new and structurally novel natural product with antimicrobial activity and, hopefully, new modes of action will be one of the ways to tackle and solve this problem. At the same time, by focusing and targeting alkaloid-containing medical plants, structural novelty with the required bioactivity is hoped to be achieved more efficiently. This is because alkaloid is believed to have diverse structures and many show a range of pharmacological activities including antimicrobial activities.

Bioactivity of Selected Plants

For the last few years, the antimicrobial properties of plants extracts and natural product have been intensively investigated as the demand for safe drugs, which has increased due to the misuse of antibiotics and an increase in immuno-deficiency. For instance, the uses of an extract of the root of *Sophora flavescense* and an extract of stem bark of *Magnolia alternifolia* as traditional medicines for microbial infections, strongly suggested that natural products are the major source of important antimicrobial agents.

Previous study by Rasadah and Houghton (1998) discovered that the crude extract from some species of Bignoniaceae that contained napthoquinones show antibacterial activities towards the Gram-positive bacteria (*Bacillus subtilis*, *Staphyloccus aureus and Pseudomonas aeruginosa*), Gram-negative bacteria (*Escherichia coli*) and yeast (*Candida albicans*). Recently, the methanolic extraction of *Apama tomentosa*, which is one of the species from Aristolochiaceae family, was discovered to exhibit highest activity against *Bacillus cereus* and *Bacillus subtilis* (Wiart et al. 2003). Lately, many researchers have good interests in studying the antimicrobial activities for controlling pathogens and toxin producing microorganisms in food of extracts and essential oil from aromatic plants.

Benkeblia (2004) had studied the antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). The essential oil extracts of these *Allium* plants exhibited marked antibacterial activity against *Staphylococcus aureus* and *S. enteritidis*) in which garlic showed the highest inhibition. For antifungal activity, *Fusarium oxysporum* showed the lowest sensitivity towards essential oil extracts, whereas *Aspergillus niger* and *Penicillium cylopium* were significantly inhibited at low concentrations. As a conclusion, essential oil of this plant can be used as natural antimicrobial additives in various products.

Most of the species from Aristolochia are widely used in medical herbs especially in China and Japan, such as *Aristolochia kaempferi*, *A. onoei*, *A. debilis*, *A. contorta* and *A. liukiuensis*. In China *Aristolochia mollissima* which is known as "xun gu feng" is a perennial shrub. The decoction and extract of root and fruit of this plant have been used in traditional medicine as analgesic, anticancer, anti malaria and anti-inflammatory agents and also for the treatment of stomach ache, abdominal pain and rheumatisme. *Aristolochia debelis*, which is known as "qing mu xiang", has direct constrictive action on blood vessels and shows inhibitory action on the heart (Wang 1983).

In India, the rhizome of *Aristolochia indica L* is used for impotence, inflammation and also as emetic, tonic and in case of malaria fever. In Burma, the leaves are used externally and internally for skin disease. In Philippines, the rhizomes is an antidote medicine in cases of poisonous bites of insects and it is also used in treating malaria fevers, irregular menses, fatigue and intestinal disorders. In Vietnam, the rhizomes are also used for malaria, fever, and dropsy and for appetite loss. In Malaysia, *Thottea grandiflora* known as Hempedu Beruang are widely used in traditional medicine practice as afterbirth tonic, antifertility, pain killer, anti-inflammatory and snake bite antidote. The decoction and extract of root from *Thottea grandiflora* can be used as traditional medicine such as after giving birth, for womb contraction and to treat the blood clot as reported by Luiza (1990) and Burkill (1935). It had been reported that the decoction of this root can be used to treat tuberculosis (Junior 1993).

The Study

UiTM Forest Reserve Jengka, which is in the district of Maran, Pahang covers an area of 0.84 square kilometers and can be classified as a secondary forest. UiTM Forest Reverse Jengka was chosen for plant selection because it has been discovered that this forest is rich with the various types of flora and fauna. From the investigation, there are at least 48 species belonging to 45 genera and 26 families in which all of them have their own medical purposes. For example, for generation, the root and the leaves of genus *Thottea sp* from Aristolochiaceae family are widely used by local people in traditional medicine practice as afterbirth tonic, anti-fertility, pain killer and snake bite antidote. Other genus from Aristolochiaceae family is *Aristolochia* and *Asarum* whereby many research activities had been done on Aristolochious plants. However,

most of the plants have not been studied chemically and pharmacologically especially *Thottea sp* although there is a great potential for the isolation of novel, naturally-occurring bioactive compound.

Thottea corymbosa, also called Akar Julong Bukit or Akar Surai, is a shrub that grows in the forest of Malaysia and Sumatera. For years it is believed that the root and the leaves of *Thottea corymbosa* can be used traditionally as a medicine such as expectorant, anti-asthmatic, anti fertility, treatment for the abdominal pain and snake bite. However, the leaves of *Thottea corymbosa* have not been studied chemically and pharmacologically. Therefore, the researchers came out with the current study of extraction the *Thottea corymbosa* leaves by using organic solvents with different polarity (petroleum ether, chloroform and methanol) and to test the antimicrobial activity of the crude extracts.

Materials and Methods

Extraction Process

Plant Material

Leaves of *Thottea corymbosa* were collected from UiTM Pahang Forest Resort. A voucher specimen (voucher no. AZ6681) was deposited in the Herbarium of UKM, Bangi (UKMB).

Extraction

The air-dried leaves of *Thottea corymbosa* (9 kg) were ground in a mill to fine powder. About 5 kg of dried ground was soaked at room temperature in petroleum ether for 48 hours. Solvent removal left dark green residue (11.5 g, 0.23%). Repeated extractions were done in chloroform for 48 hours and solvent removal gave yellowish residue (93.6 g, 1.87%). Lastly, the leaves were soaked with methanol for 36 hours and solvent removal gave brownish residue (13.7 g, 0.27%).

Assays for Anti-fungal Activity

Tested Extracts

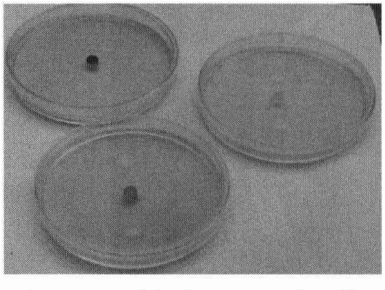
The crude extracts of petroleum ether, chloroform and methanol were tested for antimicrobial activity antifungal). They were filtered sterile through 0.45mm membrane filter before testing. Each of the solvents (petroleum ether, chloroform and methanol) was used as test control.

Microorganisms

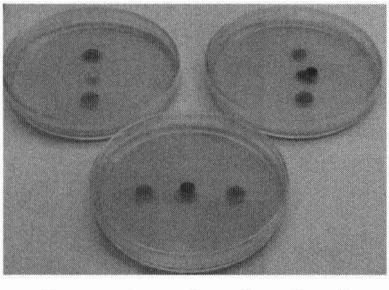
Three genus of fungi were tested for antimicrobial activity i.e. *Rhizoctonia solani*, *Sclerotium rofsii* and *Pythium sp.* the pathogens of vegetables. The fungi were isolated from diseased vegetables. The repeated test was done by using other fungi i.e. *Aspergillus niger*, *Aspergillus flavus* and *Penicillium sp.* The isolates were obtained from the building molds. The fungi were isolated on Potato Dextrose Agar (PDA) and were identified based on their morphological characteristics. They were maintained on PDA plates for further experiment.

Preparation of Inoculate

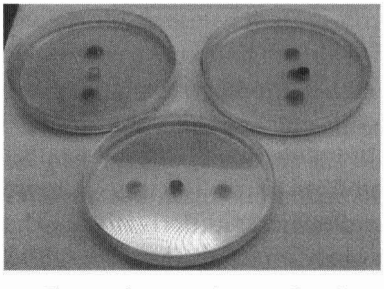
The 5 mm mycelia plug of each fungus were taken from the leading edge of 4 old day culture and placed at the centre of the Potato Dextrose Agar (PDA) plate. A drop (10 ml) of each crude extracts were dropped at periphery side of the plates. The plates were then incubated at 28°C and observed for inhibition zone after two days of incubation.



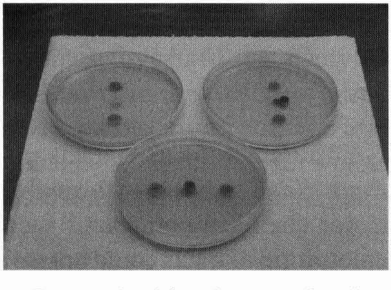
A = control (solvents vs. fungi)



B = crude methanol vs. fungi



C = crude pet-ether vs. fungi



D = crude chloroform vs. fungi

Plate 1: Dual culture plate assay of crude extracts against fungal

Results and Discussion

The in-vitro anti-fungul activities of *Thottea corymbosa* extracts (petroleum ether, methanol and chloroform) against microorganism revealed that no inhibition zone occured for the three genus of fungal tested i.e. *Rhizoctonia solani, Sclerotium rofsii* and *Pythium sp.* This revealed that there were no anti-fungal activities of crude extracts against plant pathogens. The repeated tests against *Aspergillus niger, Aspergillus flavus* and *Penicillium sp.* revealed that only chloroform extract had a little anti-fungal activities against *Penicillium sp.* (Plate 2). The negative effects of anti-fungal activities of crude extracts (petroleum ether and methanol) against tested fungi may be due to a few factors such as:

1. The non-existence of anti-fungal substances in the extracts
2. The synergistic effects of the chemical compounds

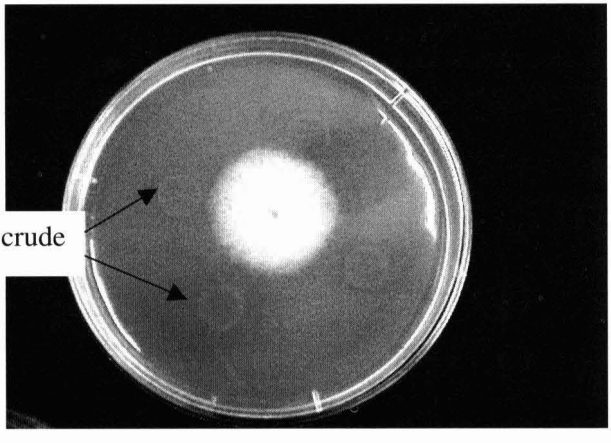


Plate 2: The Anti-fungal Activity of Chloroform Extracts Against *Penicillium sp*

The chloroform extracts give positive anti-fungal effects against *Penicillium sp* because through the chloroform extraction it gives more major chemical compound such as aristolochic acid derivatives. The chloroform extracts could not give any positive effect against *Aspergillus niger,* and *Aspergillus flavus.* This may be due to mycotoxins produced by these fungi which is normally aflatoxins. These toxins may be able to react with the chemical compound in the crude extracts. This toxin is not produced by *Penicillium sp*, but by *Aspergillus sp.*

For the synergistic effects of chemical compounds, the chloroform extracts may be can give the positive effects if the chemical compound were purified before tested to the fungi. This is because in the crude extracts, there are a few chemical compounds exist. They can synergise to one another which can reduce the effect of such chemical compound that has anti-fungal activities.

Wiart et al. (2003) revealed that Aristolochiaceae extracts could inhibit the growth of few species of bacteria. This shows that Aristolochiaceae extracts have a few anti-bacterial substances which are more effective on peptidoglycan compound that is the main component of bacterial cell walls. These anti-bacterial substances will degrade or destruct the cell walls of the bacterial cell which is mainly composed of peptidoglycan. The results of the current study revealed that the crude extracts of *Thottea corymbosa* did not have anti-fungal substances which cannot inhibit the growth of fungi. The anti-fungal substances normally contain glucanase and chitinase which can degrade or destruct the fungal cell wall that mainly consist of chitin and glucan. This shows that the crude extracts of *Thottea corymbosa* do not have any glucanase and chitinase compound.

For further study of this *Thottea corymbosa* plants, we can test the crude extracts for anti-bacterial activities and anti-tumor activities. The test can also be done by using the pure chemical isolated compound, where the chemical compound must be isolated before test to the test target.

Conclusion

The results revealed that the crude extracts of *Thottea corymbosa* have cytotoxicity effects rather than anti-fungal effects. For further study of this *Thottea corymbosa* plants, tests on the crude extracts for anti-bacterial activities and anti-tumor activities can be carried out. The test can also be done by using the pure chemical isolated compound, where the chemical compound must be isolated before test to the test target.

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SITI ZAITON MAT SO'AD, NENI KARTINI CHE MOHD RAMLI & AIZA HARUN, Faculty of Applied Sciences, Universiti Teknologi MARA Pahang. dszaiton@pahang.uitm.edu.my