

PRELIMINARY ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF
Clinacanthus nutans AND *Azadirachta indica*

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To my beloved father Mr. Goonasakaran and mother Mrs. Chandrika who have given me nothing but love and care.

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ABSTRACT

Clinacanthus nutans which belongs to the family of Acanthaceae and *Azadirachta indica* which belongs to the family of Meliaceae are often used as herbal remedies in complementary and alternative medicine. Extracts from dried leaves of *Clinacanthus nutans* and *Azadirachta indica* was screened for its antimicrobial activity and phytochemical composition. The antimicrobial activity of plant extract was first assessed by using different solvent. Chloroform, absolute ethanol, 70% ethanol and distilled water plant extracts were tested against *Salmonella enterica* serovar Paratyphi B. It was shown that chloroform *Clinacanthus nutans* extract and ethanolic *Azadirachta indica* extract showed a larger inhibition zone upon *Salmonella enterica* serovar Paratyphi B compare to other solvents. The best result producing solvent extracts were then tested upon five strains of *Salmonella* simultaneously. The strains that were subjected to test was respectively *Salmonella enterica* serovar Paratyphi C, *Salmonella enterica* serovar Typhimurium, *Salmonella enterica* serovar Weltevreden, and *Salmonella enterica* serovar Typhi ATCC and *Salmonella enterica* serovar Paratyphi B. In this test, it is shown that both plant extracts able to form zone of inhibition against the *Salmonella* strains. The enteric fever causing *Salmonella enterica* serovar Typhi ATCC showed the least inhibition with both extracts compared to the *Salmonella enterica* serovar Paratyphi B and *Salmonella enterica* serovar Paratyphi C strains. There was no significant difference in zone of inhibition against gastroenteritis causing *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Weltevreden. The qualitative phytochemical analysis was carried out using powdered leaf samples of *Clinacanthus nutans* and *Azadirachta indica* to determine the major phytoconstituents. The analysed phytochemicals are alkaloids, flavonoids, tannins, saponins and cardiac glycosides. The analysis showed the presences of all five phytoconsituents in *Azadirachta indica* leaf sample. Meanwhile for *Clinacanthus nutan*, a positive result was shown for the presence of alkaloids, tannins, flavonoids and cardiac glycosides but a negative result for the presence of saponin.

ABSTRAK

Clinacanthus nutans yang tergolong dalam famili Acanthaceae dan *Azadirachta indica* yang tergolong dalam famili Meliaceae sering digunakan sebagai ubatan herbal. Dedaun kering daripada *Clinacanthus nutans* dan *Azadirachta indica* telah dikaji untuk aktiviti antimikrob dan komposisi fitokimia. Sebagai langkah pertama, aktiviti antimikrob ekstrak tumbuhan dinilai dengan menggunakan pelarut yang berbeza. Ekstrak daripada kloroform, etanol mutlak, 70% etanol dan air suling telah diuji terhadap *Salmonella enterica* serovar Paratyphi B. Kajian ini menunjukkan bahawa ekstrak kloroform *Clinacanthus nutans* dan ekstrak etanol mutlak *Azadirachta indica* telah menghasilkan zon perencatan yang baik terhadap *Salmonella enterica* serovar Paratyphi B berbanding dengan pelarut lain. Ekstrak tersebut kemudiannya diuji ke atas lima spesies *Salmonella* iaitu *Salmonella enterica* serovar Paratyphi C, *Salmonella enterica* serovar Typhimurium, *Salmonella enterica* serovar Weltevreden, dan *Salmonella enterica* serovar Typhi dan *Salmonella enterica* serovar Paratyphi B. Ujian ini telah menunjukkan bahawa kedua-dua ekstrak tumbuhan dapat membentuk zon perencatan terhadap spesies *Salmonella* walaupun terdapat sedikit perbezaan dalam ukuran zon perencatan. Antara spesies yang menyebabkan demam enterik, *Salmonella enterica* serovar Typhi ATCC menunjukkan zon perencatan yang kecil berbanding dengan spesies paratyphi. Spesies yang menyebabkan penyakit gastroenteritis, *Salmonella enterica* serovar Typhimurium dan *Salmonella enterica* serovar Weltevreden tidak menunjukkan perbezaan yang signifikan dalam zon perencatan. Analisis kualitatif fitokimia telah dijalankan dengan menggunakan sampel serbuk dedaun *Clinacanthus nutans* dan *Azadirachta indica* untuk menentukan kehadiran fitokimia yang tertentu. Fitokimia yang dianalisis adalah alkaloid, flavonoid, tanin, saponin dan glikosida kardiak. Keputusan yang positif telah dipemerhati untuk kehadiran kesemua lima fitokimia dalam sampel dedaun *Azadirachta indica*. Manakala, bagi *Clinacanthus nutans*, keputusan positif telah dilihat untuk kehadiran alkaloid, tanin, flavonoid dan glikosida tetapi keputusan negatif bagi kehadiran saponin.

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LIST OF ABBREVIATIONS / SYMBOLS

ATCC	-	American type culture collection
CAM	-	Complementary and alternative medicine
DNA	-	Deoxyribonucleic acid
DMSO	-	Dimethyl sulfoxide
<i>et al.</i> ,	-	and friends
FeCl ₃	-	Iron(III) chloride
GCMS,	-	Gas chromatography–mass spectrometry
H ₂ SO ₄	-	Sulfuric acid
HCl	-	Hydrochloric acid
HPLC	-	High performance liquid chromatography
NCCAM	-	National Centre for Complementary and Alternative Medicine
MHA	-	Muller Hinton agar
UV-B	-	Ultra Violet B
WHO	-	World Health Organization
°C	-	Degree celcius
μL	-	Microliter
mg	-	Miligram
ml	-	Mililiter
min	-	Minute
g	-	gram

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Salmonella is a human pathogen that known to cause infection when a person ingests foods that contain a high concentration of this bacteria. Young children are much more prone to infection even with a small number of *Salmonella*. This bacteria causes gastroenteritis, enteric fever, bacteremia with or without metastatic disease, and asymptomatic carriage in host (Lennan *et al.*, 2008). In Malaysia, most of the food poisoning cases that occur at National Service Camps, school canteens, public events and etc are caused by Enterobacteriaceae such as *Samonella* (Mushaireen *et al.*, 2010). *Salmonella* infections are one of concerned cases in developing country like Malaysia.

Instead of depending on commercial antibiotics, plant extracts can be used to treat *Salmonella* infection as an alternative treatment. This is because, plants are well known for their healing activity against both biotic and abiotic stress in their natural environment. It was found that the defence mechanism of plants against these threats derived from its own secondary metabolites (Rios *et al.*, 2005). The secondary metabolites synthesized by plants include alkaloid, phenols, quinones, glycosides flavones, flavonoids, flavonols, tannins and coumarins (Malkhan *et al.*, 2012). Plant secondary metabolites are extensively exploited as an alternative antimicrobial agent

against both human and plant pathogen. The urge to have alternate antibiotic source from other resources such as plants is high now due to the lack of resource from microorganism and also the resistance of pathogen towards the existing antibiotic (Abreu *et al.*, 2012).

The *C.nutans* which locally known as Sabah Snake plant or Belalai Gajah is well known for its anti-inflammatory, anti-viral property and also as a snake bite remedy (Yoosook *et al.* 1999). The *A.indica* plant, locally known as Neem plant or pokok mambu is used due to its multiple medicinal values. Different parts of *A.indica* was proven to have active substances that has medicinal, spermicidal, antiviral, antibacterial, insecticidal, antiprotozoal, insect-repellent, and antifungal properties. They are also believed to remove toxins from the body, neutralize free radicals, and purify the blood. *A.indica* is been extensively studied for anticancer treatment, hepato-renal protective activity, and hypolipidemic effects (Shyamapada, 2011).

In order to absorb the bioactive compounds from the plant extracts, solvents play an important role. Solvent selection will determine the absorptions of hydrophilic or lipophilic compounds from the plants. Polar solvent able to absorb hydrophilic compounds, semi polar solvent able to absorb both hydrophilic and lipophilic compounds and non polar solvent absorbs lipophilic compounds. Assessing plant extract with different solvent increase the possibilities to absorb a wide range of compounds (Charmi *et al.*, 2011).

This experiment will be conducted in order to study the antimicrobial activity of *C.nutans* and *A.indica* using four different solvent against *Salmonella* strains and to identify the possible secondary metabolites involve in the antimicrobial activity. The outcome from this study will be valuable information in drug designing using natural product in future.

1.2 Problem Statement

Due to the decreasing number of new antibiotics and the increasing number of drug-resistance pathogens, an urge to find alternative antibiotics has formed (Abreu *et al.*, 2012). Naturally existing plant secondary metabolites can be a good source for antimicrobial. In fact, the usage of plant extracts to treat various diseases has been in practice among different societies since ancient times. New technologies and research are now used to validate these practices. *In-vitro* antimicrobial activity of many plant extracts such as *Ocimum sanctum* (Joshi *et al.*, 2011), *Orthosiphon stamineus* (Chun *et al.*, 2010) has given a positive outcome. Malaysia is a country with rich diversity of plants. Although many works have been conducted, yet only a fraction of total plant has been explored for the secondary metabolites and its unique usage such as antimicrobial activity (Huda *et al.*, 2007).

1.3 Scope of Study

To the best of knowledge, little study was done on *C.nutans* and *A.indica* leaves extract against different species *Salmonella*. Hence the plants will be extracted using absolute ethanol, 70% ethanol, chloroform and distilled water and will be tested against *Salmonella enterica* serovar Paratyphi B. The larger zone of inhibition forming extracts will be tested against five different strains of *Salmonella* which are respectively *Salmonella enterica* serovar Typhi ATCC, *Salmonella enterica* serovar Weltevreden, *Salmonella enterica* serovar Typhimurium, *Salmonella enterica* serovar Paratyphi C and *Salmonella enterica* serovar Paratyphi B simultaneously to analyse the antimicrobial activity. Besides, in this experiment, the powdered plant leaves will be subjected to qualitative phytochemical analysis for the presence of tannins, alkaloids, saponins, flavonoids, and cardiac glycosides.

1.4 Significant of Study

This study which is done on *C.nutans* and *A.indica* can provide valuable information on its capability to prevent growth of human pathogens such as *Salmonella*. This study can be a stepping stone for further, detail studies on the plant's antimicrobial characteristic against other pathogens. The screening for basic phytochemicals that is done in this experiment can also provide an insight of the plants bioactive compounds and other possible activities such as antiviral, antioxidant, and anti-cancerous apart from antimicrobial activity. The outcome of a detail research from these preliminary studies can also be commercialised in future.

1.5 Objectives of Study

- i. To evaluate the effect of *C.nutans* and *A.indica* extracts using four different solvent against *Salmonella enterica* serovar Paratyphi B.
- ii. To evaluate the antimicrobial activity of *C.nutans* and *A.indica* extract against five different strains of *Salmonella*.
- iii. To qualitatively analyse the presence of selective secondary metabolites of *C.nutans* and *A.indica*.

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