ANTIMICROBIAL ACTIVITY AND CYTOTOXIC EFFECTS OF *Stachytarpheta jamaicensis* (L.) Vahl CRUDE PLANT EXTRACTS

KU ANIS SHAZURA BT INDERA PUTERA

UNIVERSITI TEKNOLOGI MALAYSIA

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KU ANIS SHAZURA BT INDERA PUTERA

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Didedikasi khas buat

mereka-mereka yang disayangi,
mereka-mereka yang banyak membantu,

En Indera Putera bin Ku Man,
Pn Sharifah bt Yusuf,
Ku Mohd Ashraff bin Indera Putera
Ku Ashrul Aizat bin Indera Putera

Yang Teristimewa

Terima kasih atas sokongan kalian,

Akhirnya…..sempurna suatu perjalanan.

“ kerana sesungguhnya sesudah kesulitan itu ada kemudahan. Sesungguhnya kesulitan itu ada kemudahan .” Surah Al-Insyirah, ayat 5-6.

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ABSTRACT

Medicinal plants are known for their ability to treat diseases due to the presence of bioactive constituents such as alkaloids, phenolic compounds, saponin, tannins and terpenoids. They can be used as anti-inflammation, antioxidants, antibiotics and anti infection drugs. *Stachytarpheta jamaicensis* (L.) Vahl is one of the medicinal plants found to be useful for treatment of diseases based on its traditional usage. This plant belongs to the family of Verbenacea and can grow up to 90 to 120 cm tall. Unfortunately, less research has been done on this plant. In this research, the objectives are to screen for the presences of phytochemicals in the crude plant extract and its antimicrobial activity. Antimicrobial activity was performed using disk diffusion method. The crude extracts were tested for saponin, tannin, flavonoid, phlobatannin, coumarin, phenolic compound and terpenoids using phytochemicals screening test. It was found that the extracts contained phytochemicals such as phenolic compound, tannin, saponin, terpenoids and flavonoid but absences of phlobatannin and coumarin. From the antimicrobial test, it showed highest inhibition zone on *Pseudomonas aeruginosa* with diameter of 10.5 mm by root extracts and slight or less inhibition on the growth of *Streptococcus sp* on all three extracts compared to other types of bacteria. The root extracts are found to inhibit most of the bacteria growth other than leaves and stem extracts maybe because of the presence of most of the phytochemicals in it. Root extract can be concluded to be the best parts to be used as antimicrobial drugs. In the study for cytotoxic effect, leaves extract shows the highest inhibition on the growth of Hela cancer cells compare to root and stem extract. During incubation of 24 and 72 hours, all of the extracts showed positive results, however, during 48 hours incubation, the cell activities or growth became unstable. Hence, the extracts can only be exposed to the cancer cell line at shorter time to avoid the lost of its phytochemicals from time to time.

ABSTRAK

Pokok ubatan dikenali dengan kebolehan ia untuk mengubati penyakit disebabkan oleh kehadiran bahan bioaktif seperti alkaloid, fenolic, saponin, tannin dan terpenoids. Ia boleh digunakan sebagai anti-keradangan, antioksidan, antibiotic dan ubat antijangkitan. *Stachytarpheta jamaicensis* (L.) Vahl merupakan salah satu pokok ubatan yang diketahui amat berguna untuk menyembuhkan penyakit berdasarkan penggunaannya secara tradisional. Pokok ini berasal dari keluarga Verbenacea dan boleh membesar sehingga 90 ke 120 cm tinggi. Malangnya, hanya sedikit kajian dilakukan berkenaan pokok ini. Objektif kajian ini adalah untuk mengesan kehadiran jenis fitokimia yang terdapat dalam ekstrak tumbuhan ini serta aktiviti antibakterianya. Ujian antibakteria dijalankan menggunakan ujian cakera serapan. Ekstrak tumbuhan tersebut telah diuji untuk kehadiran saponin, terpenoids, tannin, bahan fenol, coumarin, phlobatannin dan flavonoid, menggunakan ujian fitokimia. Setelah kajian dibuat, ekstrak tersebut telah didapati mengandungi bahan fenol, tannin, saponin, terpenoid dan flavonoid tetapi tiada kesan kehadiran coumarin dan phlobatannin. Berdasarkan kepada ujian antibakteria, ia menunjukkan zon rencatan terbesar pada *Pseudomonas aeruginosa* dengan diameter 10.5 mm dan sedikit ataupun tiada zon rencatan pada pertumbuhan *Streptococcus sp*.berbanding bakteria lain. Ekstrak akar ditemui dapat menghalang pertumbuhan kebanyakan bakteria berbanding ekstrak batang dan daun disebabkan oleh kehadiran lebih banyak fitokimia di dalamnya. Ekstrak akar dapat disimpulkan sebagai bahagian terbaik dari pokok untuk digunakan sebagai ubat antibakteria. Untuk kajian kesan ketoksikan, ekstrak tumbuhan menunjukkan rencatan terbaik terhadap pertumbuhan sel Hela dibandingkan dengan ekstrak akar dan batang. Semasa inkubasi selama 24 dan 72 jam, kesemua ekstrak member kesan positif kecuali pada 48 jam inkubasi, aktiviti sel menjadi tidak stabil. Maka, ekstrak tumbuhan ini hanya boleh didedahkan pada sel untuk jangka masa yang pendek bgi mengelakkan dari kehilangan fitokimia dari masa ke masa.

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LIST OF ABBREVIATIONS

AIDS	-	Acquired Immune Deficiency Syndrome
ANOVA	-	Analysis of variance
DNA	-	Deoxyribonucleic acid
FADH	-	Flavin adenine dinucleotide (reduced form)
FAS	-	Fatty acid synthase
FRIM	-	Forest Research Institute Malaysia
kD	-	Kilo Dalton
LB	-	Luria-Bertani
MH	-	Mueller Hinton
MIC	-	Minimum Inhibitory Concentration
MTT	-	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)
NA	-	Nutrient Agar
NADH	-	Nicotinamide adenine dinucleotide (reduced form)
NADPH	-	Nicotinamide adenine dinucleotide phosphate (reduced form)
PBS	-	Phosphate buffer saline
PDA	-	Potato Dextrose Agar
RNA	-	Ribonucleic acid
USDA	-	United States Department of Agriculture
UV	-	Ultraviolet
WHO	-	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 Background information

Plants are normally being commercialized as a food-based product but they also have other important roles making them an attractive thing to be explored by the researcher. They grab the attention of the researchers by showing some medicinal properties. These medicinal plants have been used for centuries as remedies and the richest bio-resources of drugs of traditional medicinal systems in pharmaceuticals, folk medicines, nutraceuticals and synthetics drugs. Medicinal plants or herbal remedies are valuable in the treatment of various health problems (Das *et al*., 2010). Previous research conducted showed that these medicinal plants contain bioactive constituents with certain physiological that could be used in treatment of diseases (Pieme *et al*., 2006). These includes tannins, flavonoids, terpenoids, alkaloids and phenolic compounds (Hill., 1952). Besides, some of the synthetic drugs used in the medicinal field also contain the chemicals or active components obtain from these medicinal plants as an alternative due to the increase of resistance of pathogens towards frequently used drugs. Despite the modern medicines in the market, most of the plants such as *Murraya koenigii*, *Curcuma longa*, *Lawsonia inermis*, *Ficus deltoidia* and *Zingiber officinale* are extensively used in the medicinal field especially as a herbal remedies. Most of these plants extract are used as anti-inflammation, antioxidants, antimicrobial and in the treatment of cancer.

Among them all, flavonoid is the most extensively used constituents because of its properties to inhibit or kill numerous bacterial strains and also some viral enzymes (Havsteen B.H., 2002). Flavonoids are active compounds that are usually found in fruits, vegetables, seeds, stem, flowers, honey and propolis (Cushnie T.P. *et al*, 2005). Flavonoid contains substances such as flavonoles, 2-phenyl-3-hydroxy-chromones, flavones, 2-phenyl-chromones, iso-flavonoles, 3-phenyl-2-hydroxy-chromones and other compounds which have been characterized in the plants. Unfortunately, not many research focuses on *Stachytarpheta jamaicensis* (L.) Vahl. Hence, there are chances that this plant can be used as antimicrobial agents.

1.2 Problem statement

Nowadays, the production of antimicrobial drugs in pharmaceutical industries has increased due to the increase in untreatable diseases. This disease cause by microbes which have become resistant towards commonly used antimicrobial drugs. Thus, researches try to find other source of compounds which can be turned into antimicrobial drugs. Recently, attempts made to use herbal plants as one of the alternative drugs in the treatment of diseases have increased due to the impact it gives on the human health and disease prevention. Till now, there is less than thirty researches published on this plant species; *Stachytarpheta jamaicensis* (L) Vahl and mostly do not focus on the cytotoxic effect but more to its antimicrobial properties In this research, the plant extracts of *Stachytarpheta jamaicensis* (L). Vahl were determined for its antimicrobial activity and cytotoxic effect. Moreover, the research on this plant only using the leaves extract, therefore, in this study, the stem and root extract of this plant was investigated for its antimicrobial activity. Furthermore, there is less cytotoxic effect regarding this plant species.

1.3 Objective of the research

The objectives of this research are:

1. To obtain crude extracts of *Stachytarpheta jamaicensis* (L.) Vahl.
2. To detect the presence of phytochemical compounds in the crude extracts of *Stachytarpheta jamaicensis* (L.) Vahl.
3. To determine the antimicrobial activity of the crude extracts using disk diffusion technique.
4. To check for cytotoxic activities of the crude extracts of *Stachytarpheta jamaicensis* (L.) Vahl on cancer cells

1.4 Scope of the research

The study includes three important steps in which consist of preparation of the crude extract of *Stachytarpheta jamaicensis* (L). Vahl, determinations of phytochemicals content in the plant extracts and antimicrobial analysis using disc diffusion method. Methanol was used as a solvent to extract the bioactive compounds. The plant was dried and macerated into powder form before mix with methanol to produce extract. According to previous work, *Stachytarpheta jamaicensis* (L.) Vahl contains several phytochemical constituents which are important in the treatment of diseases examples ulcer, fever and diarrhea. Therefore, determination of the bioactive compounds using qualitative tests is very important to be carried out. The antimicrobial test is performed by impregnating the sterile blank disc with diluted plant extract of various concentrations and measurement of the inhibition zone. Cytotoxic activities of *Stachytarpheta jamaicensis* (L.) vahl is done by treating the Hela cancer cells using the plant extracts and check using alamar blue solution.

1.5 Significance of the research

The significance of this research is to identify the possibilities of using *Stachytarpheta jamaicensis* (L) Vahl extracts for an antimicrobial drugs based on its antimicrobial activity. Moreover, the cytotoxic activity of *Stachytarpheta jamaicensis* (L.) Vahl on cancer cells also being determine to see whether the extracts can be used in the treatment of cancer. Hence, it can act as one of the solution to treat cancer due to the fact that it is the number one cause of death worldwide.