UNIVERSITI PUTRA MALAYSIA

ANTIMICROBIAL AND WOUND HEALING ACTIVITIES OF THREE CASSIA SPECIES

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Master of Science

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DEDICATION

"Dedicated especially to Dr Nazrul Hakim Abdullah (supervisor), my parents Ismail Ibrahim and Siti Mahfuzah, Mr. Reezal Ishak whose sacrifice and support has enabled me to complete this study successfully".

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Cassia alata, Cassia fistula and *Cassia auriculata* are largely used in traditional medicine for centuries, to improve health and well being of human civilizations in rural areas of developing countries worldwide. The objective of this study is to investigate the antimicrobial and wound healing activities of all three *Cassia* species. The first two experiments were conducted to investigate the antibacterial and antifungal activities of three *Cassia* species using disc diffusion methods. Ethanol and aqueous extract of all three *Cassia* species were tested *in vitro* against the bacteria *Escherichia coli, Salmonella enteritidis, Staphylococcus aureus* and *Bacillus subtilis,* and the fungi, *Candida albicans, Candida tropicalis, Microsporum canis* and *Aspergillus fumigatus.* All three *Cassia* species were effective against *Staphylococcus aureus* and *Bacillus subtilis* in a dose dependent manner, and were not effective against *Escherichia coli* and *Salmonella enteritidis.* The results were compared with commercial antibiotics chloramphenicol (30 mg/ml), ampicilin (10 mg/ml), penicillin G (10 mg/ml), erythromycin (15 mg/ml), tetracycline (30 mg/ml)

and enrofloxacin (5 mg/ml). All three plants were only effective against the Gram-positive bacteria. The ethanol leaf extract of the plants at concentration 80 mg/ml can be compared to the commercial antibiotic, penicillin against *Bacillus subtilis*. Only *Cassia alata* and *Cassia auriculata* has exhibited antifungal activity. *Cassia fistula* has no effect against all tested fungi. The ethanol and aqueous bark extracts from *Cassia alata* was only effective against *Candida albicans*, whereas the ethanol leaf and bark extract from *Cassia auriculata* was only effective against *Microsporum canis*. When comparing the two plants against each other, we found that the plant extracts was selective and has a very narrow spectrum against the tested fungi. The ethanol leaves extracts from all *Cassia* species were chosen for the third test, which was the wound healing activity in mice. Topical application over an incised wound showed progressive infiltration of inflammatory cells, increased blood vessel formation, and enhanced proliferation of cells because of treatment with *Cassia fistula* extract and acriflavine. Wound contraction in specimens from the groups treated, respectively, with *Cassia fistula*, acriflavine and control, showed significant structural improvement when compared to *Cassia alata* and *Cassia auriculata*, in which there were no signs of healing at the end of the experiment. It can be concluded that, ethanol extracts of the leaf and bark of *Cassia alata, Cassia fistula* and *Cassia auriculata* showed antibacterial and antifungal activity which may be attributed to the presence of chemical constituent such as flavonoids, chrysophanol antharaquinones, and chrysarobin. This study also showed the promising wound healing activity of *Cassia fistula* in mice and warrants detailed experimental and clinical studies. It also provides a rationale for the use of *Cassia fistula* in preparations of traditional medicine to promote wound healing.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KEGIATAN ANTIMICROB DAN PENYEMBUHAN LUKA TIGA SPESIES *CASSIA*

Oleh

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Cassia alata, *Cassia fistula*, dan *Cassia auriculata* sudah diguna di luar bandar di seluruh dunia, selama beberapa abad dalam pengubatan tradisional untuk meningkatkan kesihatan dan kesejahteraan manusia. Objektif kajian ini ialah menyelidik kegiatan antiimikrob dan penyembuhan luka tiga species *Cassia*. Dua ujikaji dijalankan untuk menyelidik kegiatan antibakteria dan antifungus ketiga-tiga *Cassia* ini dengan mengguna kaedah resapan cakera. Ekstrak etanol dan akues untuk ketiga-tiga spesies *Cassia* ini diuji secara *in vitro* terhadap *Escherichia coli, Salmonella enteritidis, Staphylococcus aureus* dan *Bacillus subtilis*, dan terhadap fungus *Candida albicans, Candida tropicalis, Microsporum canis* dan *Aspergillus fumigatus*. Ketiga-tiga *Cassia* ini berkesan terhadap *Staphylococcus aureus* dan *Bacillus subtillis*, dan secara bersandarkan dos, dan tidak berkesan terhadap *Escherichia coli* dan *Salmonella enteritidis*. Penemuan daripada ujian ini dibandingkan dengan antibiotik komersial kloramfenikol (30 mg/ml), ampisilin (10

mg/ml), penisilin G (10 mg/ml), eritromisin (15 mg/ml), tetrasiklin (30 mg/ml), dan enroflosaksin (5 mg/ml). Ekstrak etanol daun daripada ketiga-tiga tumbuhan pada kepekatan 80 mg/ml boleh dibandingkan kepada antibiotik komersial, penisilin terhadap *Bacillus subtilis*. Hanya *Cassia alata* dan *Cassia auriculata* menunjukkan aktiviti antikulat. *Cassia fistula* tidak menunjukkan kesan terhadap kesemua kulat yang diuji. Ekstrak etanol dan akues batang daripada *Cassia alata* hanya berkesan terhadap *Candida albican*, manakala ekstrak etanol daun dan batang daripada *Cassia auriculata* hanya berkesan terhadap *Microsporum canis*. Apabila dibandingkan kedua-dua tumbuhan itu sesama sendiri, kami dapati bahawa extrak tumbuhan memilih dan mempunyai spectrum yang kecil terhadap kulat yang diuji. Ekstrak etanol daun daripada ketiga-tiga *Cassia* dipilih untuk ujian ketiga, iaitu kegiatan penyembuhan luka pada mencit. Penggunaan secara topikal kepada luka hirisan menunjukkan penyusupan sel keradangan secara progresif, dan peningkatan pemproliferatan sel yang diperlaku dengan ekstrak *Cassia fistula* dan akriflavin. Pengecutan luka dalam spesimen daripada kumpulan terperlaku dengan *Cassia fistula*, akriflavin, dan kawalan, menunjukkan pembaikkan struktur secara tererti apabila dibandingkan dengan *Cassia alata* dan *Cassia auriculata*, di mana tiada petanda yang menunjukkan bahawa berlakunya penyembuhan pada ahkir ujikaji tersebut. Apa yang boleh disimpulkan ialah, ekstrak etanol untuk daun dan batang *Cassia alata, Cassia fistula* dan *Cassia auriculata* menunjukkan kegiatan antibakteria dan antifungus spectrum luas tererti, disabitkan kepada wujudnya unsur kimia seperti flavonoid, krisofanol, antrakuinon dan krisorabin yang mungkin bertanggungjawab kepada kegiatan antimikrob tersebut. Kajian ini mengesahkan bahawa kegiatan penyembuhan luka *Cassia fistula* berpotensi dalam mencit dan adalah wajar ujikaji dan kajian klinikal yang lebih mendalam dijalankan. Kajian ini

juga menunjukkan yang penggunaan *Cassia fistula* dalam persediaan ubatan tradisional untuk merangsang penyembuhan luka adalah rasional.

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LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS

AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of variance
ALP	Alkaline phosphatase
APACHE	Acute Physiology and Chronic Health Evaluation
CNS	Central nervous system
DNA	Deoxyribonucleic acid
IBS	Institute of Bioscience
MARDI	Malaysian Agricultural and Research Development Institute
MIC	Minimum inhibitory concentration
p	Significant value
SD	Standard division
UPM	Universiti Putra Malaysia

CHAPTER I

INTRODUCTION

Medicinal plants are widely used by all sections of people either directly as folk remedies in different indigenous system of therapy or indirectly in the pharmaceutical preparation of modern medicines. Recent years have witnessed an upsurge in worldwide used of medicinal plants, plant parts and/or active ingredients interest among scientific institutions, biological research societies and in health care providers (Fransworth *et al.,* 1985).

In developing countries, particularly in the rural areas and among the urban poor, herbal medicine is, in most cases, the only form of health care. It is woven with magical-religious elements and sick persons would immediately turn to "someone who knows" and only as a last resort will consult a regular physician.

Many medical practitioners with training in pharmacology and pharmacognosy are well aware of the number of modern therapeutic agents that have been derived from tropical forest species. In fact, over 120 pharmaceutical products currently in use are plant-derived; some 75% of these discovered by examining the use of these plants in traditional medicine (Fransworth *et al.,* 1985).

Herbs have been used for centuries to improve health and well being of civilization (Lindley, 1981). Initially, the term "herb" only applied to non-woody plants, but today, "herb" refers to any part of a plant used for medicinal or flavouring purposes. An "herb" may be a fruit, a bark, a flower, a leaf or a root as well as non-woody plant (Usher, 1974).

It is estimated that fifty-six percent of low-income world's population use herbal medicine and supplementation for their primary health care (Planta *et al.*, 2000).

Respiratory infections, diarrhea, fungal/bacterial infections, diabetes and malaria are among the common health problems occurring in the rural communities in tropical developing countries. Numerous tropical medicinal plants are used traditionally and have been shown *in vivo* to possess biological activities against these diseases (Pinn, 2000).

Antimicrobial agents are the synthetic and natural compounds/drugs to suppress growth of microorganism. A bactericidal agent kills bacteria, whereas a bacteriostatic agent inhibits their growth but does not kill them (Levinson and Jawetz, 1998).

Bacteria are minute, unicellular, plant-like, microscopic organisms, which differ from true plants in that they lack chlorophyll. They reproduce by binary fission. They are widely distributed in soil, air, water, and milk, on the surface of

fruits and vegetables, and in various parts of the body such as the alimentary canal and skin (Davies, 1994).

Gram-positive bacteria are prokaryotic cell whose cell wall consists chiefly of peptidoglycan and lack of the outer membrane of gram-negative cells. Gram-positive bacteria is, bacteria that take the initial stain of the Gram stain and are not decolourised, so that they appear purple (Madigan *et al.,* 1997). Two common bacteria, which cause serious diseases in human, were chosen for this study, *Staphylococcus aureus* and *Bacillus subtilis.*

Gram-negative bacteria are prokaryotic cell whose cell wall contains relatively little peptidoglycan but has an outer membrane composed of lipopolysaccharide, lipoprotein, and other complex macromolecules. The lack of peptidoglycan, makes the bacteria loses the initial stain of the Gram stain, is decolourised, and takes the colour of the final stain; red (Madigan *et al.,* 1997). Two common Gram negative bacteria, which cause serious diseases in human, were chosen for this study, *Escherichia coli* and *Salmonella enteritidis*.

Fungi are nonphototrohic eukaryotic microorganisms that contain rigid cell walls. Fungi can be differentiated from prokaryotes by the fact that fungal cells are usually much larger and contain a nucleus, vacuoles, and mitochondria, which are typical of eukaryotic cells. There are three groups of fungi with major practical importance, the moulds, yeast and mushrooms. In this study, only the moulds and yeast are used (Madigan *et al.,* 1997).

The habitats of fungi are quite diverse. Some are aquatic, living primarily in fresh water, and a few marine fungi are known. Most fungi have terrestrial habitats, in soil or on dead plant matter, and these types often play crucial roles in the mineralization of organic carbon in nature. A large number of fungi are parasites of terrestrial plants, a few fungi are parasitic on animals, including humans, although in general fungi are less significant as animal pathogens that are bacteria and viruses (Madigan *et al.,* 1997).

Moulds are filamentous fungi. They grow as long filament (hyphae) and become mat (mycelium). They are widespread in nature and are commonly seen on stale bread, cheese, or fruit. *Aspergillus fumigatus* and *Microsporum canis* are used in this study. Both moulds are from clinical isolates.

Yeast are unicellular (single cells) fungus and grows asexual budding, most of them are classified with the Ascomycetes. Yeast usually flourishes in habitats where sugars are present, such as fruits, flowers, and the bark of trees. A number of yeast species live symbiotically with animals, especially insects, and a few species are pathogenic for animals and humans. The yeast used in this study is *Candida albicans* and *Candida tropicalis*. Both *Candida* species are from clinical isolates.

Wound healing is a complex sequence of events is initiated by the stimulus of injury to tissues. A positive stimulus may result from the release of some factors by the wounding of tissues (Allison, 1992). This sequence of physiologic events occurs by a process of connective tissue repair. These events involve the migration, proliferation, adhesion and differentiation of the epithelial cells (Raghow, 1994).

Objective of the study

1) To evaluate the effects of ethanol and aqueous of leaves and barks extracts from *Cassia alata*, *Cassia fistula* and *Cassia auriculata* against several bacteria *in vitro*.
2) To evaluate the effects of ethanol and aqueous of leaves and barks extracts from *Cassia alata, Cassia fistula* and *Cassia auriculata* against fungi *in vitro*.
3) To determine the ethanol leaves extracts of *Cassia alata*, *Cassia fistula* and *Cassia auriculata* in promoting wound healing in mice.

CHAPTER II

LITERATURE REVIEW

The plants

Cassia is an herbaceous plant belonging to Caesalpiniaceae tribe of Leguminose family. *Cassia alata*, *Cassia fistula* and *Cassia auriculata* are largely used in traditional medicine for centuries, to improve health and well being of civilizations in rural areas of developing countries worldwide. All three plants continued to be used as major source of medicine in primary health care. Their usage varies around the world and concentrated on treatment of skin diseases (ringworm) externally (Usher, 1974).

The extracts of *Cassia alata*, *Cassia fistula* and *Cassia auriculata* have similar composition, with a slight variation in the amount of their active components and each is purported to have different therapeutic properties. However, little has been done to compare the effectiveness of the three species (Percival, 2000).

Although many of the active components of *Cassia* have been identified (Table 1), their mechanism of action is not completely known nor is the bioavailability, relative potency or the synergistic effects of the active compounds known (Giron, 1991).