UNIVERSITI PUTRA MALAYSIA

IN- VITRO PHARMACOLOGICAL PROFILE OF PARTIALLY PURIFIED LEAVES OF ALSEODAPHNE PERAKENSIS

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IN-VITRO PHARMACOLOGICAL PROFILE OF PARTIALLY PURIFIED LEAVES OF *ALSEODAPHNE PERAKENSIS*

By

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

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Specially Dedicated

To

Amarachi Ikpegbu Okechukwu Nwabueze

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements of the degree of Master of Science

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Alseodaphne perakensis (AP) is a moderately sized tree that is widely distributed throughout Peninsular Malaysia. There are no reports of this tree in traditional folk medicine although tests have shown its leaves are rich in alkaloids. Initial experimental work carried out on this species led to the isolation of a major compound *N*-methyl-2,3,6-trimethoxymorphinandien-7-one, an alkaloid with a morphinandienone skeleton which is similar in structure with *O*-methylflavinanthine; and a minor polar alkaloid *N*-methyl-2,3,6-trimethoxymorphinandien-7-one-*N*-oxide. Another compound 7-hydroxy-2,3,6-trimethoxy phenantherenel has also been isolated. All these three compounds isolated from AP have no reported biological activity. However, the alkaloid *O*-methylflavinanthine has been previously isolated from other plants and its analgesic properties has been reported by other researchers.

In this study, the crude methanol extract (CMLE) and semi-pure alkaloids were extracted by steeping the air-dried leaves of AP in methanol for 48hrs. The solvent methanol containing CMLE were evaporated using the rotary evaporator at 45°C leaving a blackish viscous CMLE. The alkaloid in CMLE were extracted using the sulphuric acid, sodium carbonate, and methylene chloride (DCM) to obtain DCM 'A' and DCM 'B' and de-alkaloid fractions (residue after alkaloid extraction). Alkaloid test (Meyer's reagent) done on these extracts CMLE and DCM 'A' and DCM 'B' fractions confirmed the presences of alkaloid; the residue after alkaloid extraction tested negative for alkaloid.

These extracts CMLE, DCM 'A' and DCM 'B' from the leaves of AP were tested on stimulated and unstimulated guinea pig ileum (GPI), rat vas deferentia (RVD) and mouse vas deferentia (MVD) preparations.

The CMLE (100 µl, 0.1 g/ml) inhibited the electrically induced twitches on the GPI preparation. On its own, CMLE has no effect on the unstimulated GPI. Contractions induced by histamine and acetylcholine on the unstimulated GPI preparation were antagonised in a non-competitive manner by CMLE (100 µl 0.1 g/ml). DCM 'A' (100 µl 0.1 g/ml) was also found to inhibit the electrically induced twitches on the GPI preparation. On its own, DCM 'A' had no effect on the unstimulated GPI. DCM 'A' also antagonised the contraction induced by histamine and acetylcholine on the unstimulated GPI in a non-competitive manner.

The CMLE (100 µl 0.1 g/ml) inhibited the electrically induced twitches on the RVD preparation. On its own, CMLE had no effect on the unstimulated RVD.

Contractions induced by phenylepherine on the unstimulated RVD preparation was antagonised in a competitive manner by CMLE (100 µl 0.1 g/ml). Like CMLE, DCM 'A' (100 µl 0.1 g/ml) inhibited the electrically induced twitches on the RVD preparation. On its own, DCM 'A' had no effect on the unstimulated RVD. DCM 'A' also antagonized contractions induced by phenylepherine on the unstimulated RVD. Like phenylepherine antagonist phentolamine, both CMLE and DCM 'A' competitively inhibited contraction induced by phenylepherine on RVD.

CMLE and DCM'A' inhibited the electrically induced twitch on the stimulated MVD; they did not have any effect on the unstimulated MVD. The inhibition by CMLE and DCM'A' fraction on the stimulated MVD was reversed by naloxone.

DCM 'B' fraction did not have any effect on the stimulated GPI, RVD and MVD preparation. It also did not have any effect on the unstimulated RVD and MVD, however, DCM 'B' fraction induced contractions in a dose dependent manner on the unstimulated GPI. These contractions were antagonised competitively by mepyramine. The dealkaloid (dAK) fractions did not show any physiological effect on the stimulated and unstimulated GPI, stimulated and unstimulated RVD and stimulated and unstimulated MVD.

CMLE and DCM 'A' fractions from the leaves of AP seem to possess; antihistaminergic, antimuscarinic, antiadrenergic, morphine-like activity. DCM 'B' fraction exhibited histaminergic activity.

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

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Oleh

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Alseodaphne perakensis (AP) merupakan pokok sederhana saiz yang tersebar luas di seluruh Peninsular Malaysia. Walaupun terdapat kajian yang menunjukkan daunnya kaya dengan alkaloid tetapi tiada laporan mengenai sifat perubatan traditionalnya dilaporkan. Sebatian N-methyl-2,3,6, trimethoxymorphinan-7-one (O-methylflavintine), iaitu alkaloid yang mempunyai struktur morphian dan satu minor alkaloid N-methyl-2,3,6, trimethoxymorphinan-7-one-N-oxide yang diisolasi dalam kajian awal telah menarik minat penyelidik dalam kajian ini. Sebatian lain iaitu 7-hydroxy-2,3,6, trimethoxy phenantherenel juga telah diisolasikan dan tidak ada laporan mengenai aktiviti biologi bagi sebatian ini. Namun begitu, alkaloid O-methylflavintine telah diisolasi dari tanaman lain and kandungan analgesi telah dilaporkan oleh ahli penyelidik lain.

Dalam kajian ini, krude metanol ekstrak (CMLE) dan alkaloid separuh tulen telah diekstrak daripada daun AP yang kering dalam methanol selama 48 jam. Pelarut

methanol yang mengandungi CMLE telah dievaporasi dengan mengguna evaporator pemuntar pada suhu 45 °C dan meninggal CMLE yang lekat dan berwarna kehitaman. Alkaloid dalam CMLE diekstrakkan dengan mengguna asid sulfurik, sodium carbonate dan metilena klorida (DCM) untuk mendapat DCM 'A' dan DCM 'B' serta sebatian de-alkaloid (sisa selepas pengekstrakan alkaloid). Ujian alkaloid (reagen Meyer) dilakukan terhadap ekstrak CMLE, DCM 'A' dan 'B' untuk mengesan kehadiran alkaloid. Sisa selepas ujian alkaloid telah diuji negatif kehadiran alkaloid.

Ekstrat CMLE, DCM 'A' dan DCM 'B' daripada daun AP telah diuji pada unjuran musel ileum dari guinea pig (GPI), "rat vas deferentia" (RVD) dan "mouse vas deferentia" (MVD) yang dirangsangkan dan tidak dirangsangkan.

CMLE (100 µl 0.1 g/ml) telah menghalang kekenjangan elektrik pada GPI yang dirangsangkan tetapi tidak ada kesan pada GPI yang tidak dirangsangkan. Kejutan yang diransangkan oleh histamine and acetylcholine pada GPI yang tidak distimulasi adalah disebabkan oleh kesan antagonis bukan pesaing CMLE (100 µl 0.1 g/ml). DCM 'A' (100 µl 0.1 g/ml) juga menghalang kekenjangan elektrika pada GPI yang dirangsangkan tetapi tidak ada kesan pada GPI yang tidak dirangsangkan. DCM 'A' juga mengantagonis kejutan yang dirangsangkan oleh histamine dan acetylcholine pada GPI yang tidak diransangkan.

CMLE (100 µl 0.1 g/ml) menghalang kekenjangan elektrika pada RVD yang dirangsangkan tetapi tidak ada kesan pada RVD yang tidak dirangsangkan. Kejutan yang diransangkan oleh pheylepherine pada RVD yang tidak distimulasi adalah

disebabkan oleh kesan antagonis bukan pesaing CMLE (100 µl 0.1 g/ml). Seperti CMLE, DCM 'A' (100 µl 0.1 g/ml) juga menghalang kekenjangan elektrika pada RVD yang dirangsangkan tetapi tidak ada kesan pada RVD yang tidak dirangsangkan. DCM 'A' juga mengantagonis kejutan yang dirangsangkan oleh phenylepherine pada RVD yang tidak diransangkan. Seperti phenylepherine antagonis phentolamine, kedua-dua CMLE dan DCM 'A' menghalang kejutan yang diransangkan oleh phenylepherine.

CMLE dan DCM 'A' menghalang kekenjangan elektrika yang dirangsangkan oleh MVD tetapi tidak ada kesan pada MVD yang tidak diransangkan. Naloxone juga memberi kesan yang sama.

DCM 'B' tidak memberi sebarang kesan terhadap GPI, RVD dan MVD yang dirangsangkan. Ia juga tidak memberi sebarang kesan terhadap RVD dan MVD yang tidak dirangsangkan. Akan tetapi, DCM 'B' menunjukkan kejutan pada GPI yang dirangsangkan bergantung sukatan yang diberikan. Kejutan ini diantagonis oleh mepyramine secara bersaingan. Dealkaloid (dAK) tidak menunjukkan sebarang kesan fisiologi pada GPI, RVD dan MVD yang dirangsangkan dan tidak dirangsangkan.

CMLE dan DCM 'A' daripada daun AP menunjuk aktiviti antihistaminergik, antimuscarinik, antiadrenergik, "morphine-like". DCM 'B' menunjukkan aktiviti histaminergik.

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LIST OF ABBREVIATIONS

ACh	- Acetylcholine
ANS	- Autonomic nervous systems
AP	- *Alseodaphne perakensis*
Atr	- Atropine
CMLE	- Crude methanol extract
CNS	- Central nervous systems
DA	- Dopamine
dAK	- dealkaloid
DCM	- Methylene chloride
DCM'A'	- Semipure Methylene Chloride A
DCM'B'	- Semipure Methylene Chloride B
DMSO	- Dimethylsuphoxide
FDA	- Food and Drug Administration
GABA	- Gama-amino-butyric acid
GPI	- Guinea pig ileum
His	- Histamine
Mep	- Mepyramine
Mor	- Morphine
MVD	- Mouse vas deferentia
Nal	- Naloxone
PANS	- Parasympathetic nervous system
PCP	- Phencyclidine
Pht	- Phentolamine

Phy	- Phenylepherine
POMC	- Pro-opiomelanocortin
RVD	- Rat vas deferentia
TCM	- Traditional Chinese Medicine
TLC	- Thin layer chromatography

CHAPTER I

INTRODUCTION

The relationship between man and plant has been very close throughout man's civilisation and the development of human cultures. Through man's history, botany and medicine were for all practical purposes, synonymous fields of knowledge. Plants have been used by different cultures for many centuries in the treatment of human disorders and pains. In the rural areas, people resort to fresh plants collected in their vicinity, whereas the urban people resort to special stores for their supply of herbs. Traditional medicine practice has long been based on religious belief and the use of plants as complementary medicine. The use of plants for the treatment of disorders is as old as the world itself (Adam and Eve used plants for the treatment of disorders). A review of the practice of ancient traditional medicine in different parts of the world reveals the important role of plants as a source of medicine for controlling pains.

Among the ancient Chinese, the term Chinese medicine has a dual implication. It refers both to the complete medical systems prevalent in contemporary China and indigenous medicine or Traditional Chinese Medicine (TCM). The three complete medical systems in China include: the traditional, the western and integrated medicine. TCM combines fighting against disease, keeping fit, and seeking longevity. It was created by all nationalities of the Chinese people and is the synthesis of all their medical systems. For historical reasons, TCM has been applied exclusively to the indigenous medical system created by the Han nationality.

(yellow Emperor's Inner Canon), Shennong Bencao Jing (Divine Husbandry's Classic of Herbology), Nan jing (classic of Questioning), Shanghan Lun (On Diseases Due to Cold Evil) and Jingui Yaolue (Synopsis of Golden Chamber). These lay the foundation for clinical science with definite treatment principles and diagnostics. Shennong's classic of herbology, presented many specific effective remedies, it initiated the theoretical basis of drug use, as well as collection, preservation, compounding, and method of administration. Therapeutic effects of specific drugs, such as *rhei* for catharsis, coptis root for asthma, seaweed for goiter and mecury for scabies.

The science and art of Ayurveda are integral parts of the cultural heritage, which is preserved, fostered, and promoted in India and its neighboring countries for the preservation and promotion of positive health and the prevention and cure of disease. The practice of Ayurvedic medicine has been in existence before (600BC) Health according to Ayurvenda is not only freedom from disease. According to Susruta, one of the great early practitioners, it is a state of the individual where, in addition to harmony among functional units, digestive and metabolic mechanisms, structural elements and waste products, a person should also be in an excellent state of the spirit, senses and mind. In Ayurvedic medicine, drugs of vegetable origin, animal products, and metals, minerals, gems and semi-precious stones are used for therapeutic purposes. These are used in their natural form or processed in order to obtain their whole extract or to make them non-toxic, palatable, and therapeutically more potent. Different parts of these drugs, like alkaloids, glucosides, and other active principles are not extracted for therapeutic use. According to Ayurvedic medicine, every drug has therapeutically useful parts that may produce toxic effects

if used alone. The same drug, however, contains other parts that counteract these adverse effects. Therefore, the use of whole drug is emphasised, and no isolation section or synthetic chemicals are used.

In Africa, for a millennium, traditional medicine has been an integral part of African culture. African traditional medicine represents the sum of the people's medicinal knowledge as well as beliefs, skills, and practices used in diagnosing, preventing, or eliminating a physical, mental, or social disequilibrium. It is based on practical experiences and observations, which have been handed down from one generation to another. African traditional medicine practice involves several types of therapeutics system, each with a distinctive approach to diagnosis therapy. They can be broadly categorised into those which are essentially "secular" these includes herbalist sometimes referred to as traditional pharmacist, they employ skills passed on from one to another generation and those which are "sacred" that is those which involve spiritual models of healing.

The practice of herbalism may be inherited from parents; it may be motivated by the desire for herbal knowledge and treatment. Some obtain the know-how through dreams or visions; others are motivated by the desire to make a living from the sale of herbal preparations. Herbalists are, therefore a mixed group of traditional health practitioners without any standardised training. While some do receive long periods of apprenticeship, through direct instruction, observation, and collection of herbs and other ingredients, many of them have little or no formal training.

In Thailand villages one finds people referred to as traditional doctors (moo phaen booraan), who use mainly herbs (samunphraj) to cure diseases, a practices has been in existence for more than 100 years. The sum of the knowledge and practices of all these experts is called traditional Thai medicine. There exist many local and individual variations within Thailand both in the way diseases are diagnosed and in the way plants and other materia medica are used in curing process. Traditional Thai medicine resembles that of its neighboring countries Burma, Laos, and Cambodia. Traditional Thai medicine contains knowledge about the identification of plants (and minerals and animals components) and their curing properties, about the diagnosis, cause, and development of disease, about prescriptions, and about the relevant incantations and ceremonies.

The whole world culture touches on the importance of plants in the remedy of diseases and pains. As the development of science and technology progresses, there became new approach to the use of plants and natural product. The extraction and isolation of active component of plant has been made possible due to the invention of chromatographic technique and mass spectrometer to mention but a few. As a result of the progress in natural product, today greater importance has been given to plants and herbs, not only by botanies but also by chemists, biochemists and pharmacologists as sources of some bioactive natural product. Plants have been the raw materials for intensive and extensive research in plant natural products and chemical synthesis. Great interest has been given to herbal medicine by researchers especially researchers in the United State of America. The creation of National Center for Complementary Medicine a research body that base their research on the development of herbal therapies attest to this fact.

Many compounds extracted from different plants have been developed into potential drug. One of the earliest breakthrough in search for bioactive compounds from plants were the isolation of morphine from a plant called poppy (*Papaver somniferum*). Morphine, which is the major active component in the poppy plant, is a painkiller. Following the discovery of morphine, many other important drugs have been developed from the plant. Atropine, an anticholinergic was extracted from *Atropa belladonna*, hyosine was isolated from *Datura stramonium*, muscarine from *Amanita muscaria*. The cardiac glycosides belong to the steroid glycosides present in the *Strophanthus* genus, the foxglove, *Digitalis purpurea*, and in the lily of the valley *Convallaria majalis*.

Camptothecin extracted from the tree *Camptotheceaea acuminita has* been developed as a drug. Two drug preparations from this plant were approved in 1996 by Food and Drug Administration (FDA) United States of America (USA). These drugs are marketed under the registered trademarks Hycamptin® and Camptoser®. These anti-tumor drugs are used against ovarian and corecteal cancer respectively (Josephson, 2000). Ergot alkaloids extracted from *Claviceps purpurea*, is currently in use as therapeutic agents as antimigraine treatments dihydroergotamine and *ergotamine*, the oxytocic agent *ergometrine* (including muscular contraction of the uterus), and the vasodilator (and hence blood-pressure depressant) dihydroergocristine. α - *Dihydroergocryptine* is used against Parkinson's disease. Alloferin® effective neuromuscular receptor blockers, which inhibit the transmission of neurosignals to the muscles at the synapses (Hesse, 2002).