UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICALS AND BIOACTIVITIES OF Curcuma mangga VALETON AND VAN ZIJP., Boesenbergia prainiana (KING EX BAKER) SCHLTR. AND Bauhinia thonningii SCHUMACH. & THONN.

HALIMATUL SAADIAH BT MOHAMMAD NOOR

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MASTER OF SCIENCE
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By

HALIMATUL SAADIAH BT MOHAMMAD NOOR

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

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

PHYTOCHEMICALS AND BIOACTIVITIES OF *Curcuma mangga* VALETON AND VAN ZIJP., *Boesenbergia prainiana* (KING EX BAKER) SCHLTR. AND *Bauhinia thonningii* SCHUMACH. & THONN.

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February 2014

Chairman: **Mohd Aspollah Bin Hj. Sukari, PhD**
Faculty: **Science**

Phytochemical and bioactivity studied on three medicinal plants, *Curcuma mangga* Val. and Van Zijp., *Boesenbergia prainiana* (King ex Baker) Schltr. and *Bauhinia thonningii* Schum. were carried out. *Curcuma mangga* Val. and Van Zijp. and *Boesenbergia prainiana* (King ex Baker) Schltr. belong to the family of Zingiberaceae while *Bauhinia thonningii* Schum. belongs to the family of Cesalpiniaceae. The chemical constituents of these plants were isolated using chromatographic methods while the structure of the compounds were elucidated using various spectroscopics methods including infrared (IR), mass spectrometry (MS) and nuclear magnetic resonance (^{1}H, ^{13}C and 2D NMR). The crude extracts and isolated compounds were screened to biological activity studies which were cytotoxic, antioxidant, total phenolic content, antimicrobial and antifungal.

The isolation work on rhizomes of *Curcuma mangga* have yielded six compounds which were identified as curcumin **(4),** demethoxycurcumin **(5),** curcumol **(51)**, curdione **(52)**, zederone **(53)** and β-sitosterol **(22)**. Curcumol **(51)**, curdione **(52)** and zederone **(53)** are reported to be first isolated from this species. For *Boesenbergia prainiana*, phytochemical studies were first reported on isolation of four compounds which were stigmasterol **(54),** lupeol **(55)**, lupenone **(56)** and β-sitosterol **(22)**. *Bauhinia thonningii* afforded three compounds elucidated as 9-hydroxytridecyl decosanoate **(57)**, betulinic acid **(58)** and friedelin **(59)** which also have never been reported previously on this species.

Crude extracts and isolated compounds were subjected to cytotoxic screening against five human cancerous cell lines; promyelocytic leukemia (HL-60), breast cancer (MCF-7), colonic cancer (HT-29), cervical cancer (HeLa) and T-lymphoblastic (CEM-SS) using MTT assay. Hexane, chloroform, ethyl acetate and methanol extracts of rhizomes of *Curcuma mangga* were active against CEM-SS cell line with IC_{50} value ranging from 11.35 to 15.41 µg/mL. This is the first report on cytotoxic activities towards CEM-SS cell line. The hexane and chloroform extracts were also active against MCF-7, HeLa and

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HT-29 cell line with IC_{50} values ranging from 5.75 to 17.9 µg/mL. Isolated compounds, **4** and **5** exhibit promising activities against all selected human cancerous cell. IC_{50} values of Curcumin **(4)** and demethoxycurcumin **(5)** against HL-60, MCF-7, HeLa and CEM-SS were ranging from 3.14 to 11.04 µg/mL. Furthurmore, curcumol **(51)** was strongly active against MCF-7 and HeLa cell lines with IC_{50} values of 6.84 and 1.25 µg/mL while curdione **(52)** was active against HT-29 cancer cell line with IC_{50} of 9.08 µg/mL. In addition, zederone **(53)** was strongly active against MCF-7 cell line with IC_{50} of 3.43 µg/mL. For *Boesenbergia prainiana*, hexane and chloroform extracts were active against MCF-7 cell line with IC_{50} of 17.54 and 18.26 µg/mL. As for *Bauhinia thonningii*, hexane, chloroform, ethyl acetate and methanol extracts were strongly active against CEM-SS cell line with IC_{50} value ranging from 9.21 to 11.12 µg/mL. These cytotoxic properties were also reported for the first time from this species.

For antioxidant assay, chloroform, ethyl acetate and methanol extract of *Curcuma mangga* show strong antioxidant effect with IC_{50} values of 40.58, 30.17 and 24.97 µg/mL, respectively while no antioxidant effect was observe on all crude extracts of *Boesenbergia prainiana.* For *Bauhinia thonningii,* ethyl acetate and methanol extracts show strong antioxidant effect with IC_{50} values of 40.58 and 24.97 µg/mL. Isolated compounds of *Curcuma mangga*, curcumin **(4)** and demethoxycurcumin **(5)** showed strong antioxidant activity with IC_{50} values of 26.67 and 31.52 µg/mL respectively. Total phenolic content (TPC) determination indicated chloroform, ethyl acetate and methanol extracts of *Curcuma mangga* have high TPC followed by ethyl acetate and methanol extracts of *Bauhinia thonningii*.

In antimicrobial screening test, methanol extract of *Curcuma mangga* exhibited remarkable antimicrobial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Bacillus subtillis*. For *Boesenbergia prainiana*, and *Bauhinia thonningii,* its crude extracts indicated low effect against MRSA, *Bacillus subtillis* and *Pseudomonas aeruginosa.* Antimicrobial activity studies have never been reported previously on *Boesenbergia prainiana* and *Bauhinia thonningii*.

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

FITOKIMIA DAN BIOAKTIVITI *Curcuma mangga* VALETON DAN VAN ZIJP., *Boesenbergia prainiana* (KING EX BAKER) SCHLTR. DAN *Bauhinia thonnigii* SCHUMACH. & THONN.

Oleh

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Febuari 2014

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Tiga jenis tumbuhan yang digunakan untuk tujuan perubatan iaitu *Curcuma mangga* Val. and Van Zijp., *Boesenbergia prainiana* (King ex Baker) Schltr. dan *Bauhinia thonningii* Schum. telah dikaji. *Curcuma mangga* Val. and Van Zijp. dan *Boesenbergia prainiana* (King ex Baker) Schltr. tergolong dalam famili Zingiberaceae manakala *Bauhinia thonningii* Schum. tergolong dalam famili Cesalpiniaceae. Hasil sebatian daripada pokok-pokok ini dipencilkan menggunakan teknik kromatografi. Stereokimia sebatian ini telah ditentukan menggunakan pelbagai teknik spektroskopi termasuk inframerah (IR), spektrometri jisim (MS) dan juga resonan magnet nukleus (^{1}H, ^{13}C dan 2D NMR). Ekstrak mentah dan juga hasil sebatian telah dijalankan ujian sitotoksik, antioksida, jumlah kandungan fenol, dan juga antimikrob dan antifungal.

Kerja pemencilan yang dijalankan ke atas *Curcuma mangga* menghasilkan enam sebatian yang dikenalpasti sebagai curcumin **(4),** demethoxycurcumin **(5),** curcumol **(51)**, curdione **(52)**, zederone **(53)** dan β-Sitosterol **(22)**. Curcumol **(51)**, curdione **(52)** dan zederone **(53)** adalah yang pertama berjaya diperolehi daripada spesies ini. Empat sebatian yang pertama dicatatkan diperolehi daripada *Boesenbergia prainiana* iaitu stigmasterol **(54),** lupeol **(55)**, lupenone **(56)** dan β-Sitosterol **(22)**. *Bauhinia thonningii* pula berjaya mengasingkan tiga sebatian dikenalpasti sebagai 9-hydroxytridecyl decosanoate **(57)**, betulinic acid **(58)** dan friedelin **(59)** yang belum pernah dicatatkan dalam spesies ini.

Semua ekstrak dan sebatian yang diperolehi telah dijalankan ujian sitotoksik terhadap lima jenis sel kanser manusia iaitu sel kanser leukimia (HL-60), sel kanser payudara (MCF-7), sel kanser kolon (HT-29), sel kanser servik (HeLa) dan sel kanser T-lymphoblastic (CEM-SS). Ekstrak heksana, kloroform, etil asetat dan metanol daripada akar *Curcuma mangga* adalah aktif terhadap sel kanser T-limfoblastik (CEM-SS) dengan nilai IC_{50} dalam julat 11.35 hingga 15.41 µg/mL. Ini adalah catatan aktiviti sitotoksik yang pertama terhadap sel CEM-SS. Ektrak heksana dan kloroform juga aktif terhadap sel MCF-7, HeLa dan HT-29 dengan nilai IC_{50} antara 5.75 hingga 17.9 µg/mL.

Hasil sebatian **(4)** dan **(5)** adalah sangat aktif terhadap semua sel kanser yang dipilih. Nilai IC_{50} Curcumin **(4)** dan demethoxycurcumin **(5)** terhadap HL-60, MCF-7, HeLa dan CEM-SS adalah antara 3.14 to 11.04 µg/mL. Seterusnya, Curcumol **(51)** adalah sangat aktif terhadap sel MCF-7 dan HeLa dengan nilai IC_{50} 6.84 dan 1.25 µg/mL. Curdione **(52)** aktif terhadap sel HT-29 dengan nilai IC_{50} of 9.08 µg/mL. Tambahan pula, zederone **(53)** adalah sangat aktif terhadap sel MCF-7 dengan nilai IC_{50} 3.43. Untuk *Boesenbergia prainiana* pula, ekstrak heksana and kloroform adalah aktif terhadap sel MCF-7 dengan IC_{50} 17.54 dan 18.26 µg/mL. Untuk *Bauhinia thonningii*, ekstrak heksana, kloroform, etil asetat dan metanol adalah aktif terhadap sel CEM-SS dengan nilai IC_{50} antara 9.21 hingga 11.12 µg/mL. Catatan aktiviti sitotoksik ini adalah yang pertama terhadap species *Bauhinia thonningii.*

Untuk ujian antioksida, ekstrak kloroform, etil asetat dan metanol daripada *Curcuma mangga* menunjukkan kesan antioksida yang tinggi dengan IC_{50} 40.58, 30.17 dan 24.97 µg/mL, manakala tiada kesan antioksida daripada semua ekstrak *Boesenbergia prainiana.* Untuk *Bauhinia thonningii,* ekstrak etil asetat and metanol menunjukkan kesan antioksida yang tinggi dengan IC_{50} 40.58 dan 24.97 µg/mL. Sebatian daripada *Curcuma mangga,* curcumin **(4)** and demethoxycurcumin **(5)** menunjukkan aktiviti antioksida yang tinggi dengan nilai IC_{50} 26.67 dan 31.52 µg/mL. Dalam penentuan jumlah kandungan fenol (TPC), ekstrak kloroform, etil asetat and metanol daripada *Curcuma mangga* mengandungi jumlah kandungan fenol yang tinggi diikuti oleh ekstrak etil asetat dan metanol daripada *Bauhinia thonningii*.

Dalam ujian antimikrob dan antifungal, ekstrak metanol daripada *curcuma mangga* menunjukkan aktiviti yang memberansangkan terhadap Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Bacillus subtillis.* Bagi *Boesenbergia prainiana* dan *Bauhinia thonningii* ekstraknya menunjukkan kesan yang lemah terhadap antimikrob *MRSA, Bacillus subtillis* dan *Pseudomonas aeruginosa.* Ujian antimikrob terhadap spesies *Boesenbergia prainiana* dan *Bauhinia thonningii* belum pernah dicatatkan sebelum ini.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

Declaration by graduate student

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
δ	Chemical shift in ppm
^{13}C	Carbon-13
$CHCl_3$	Chloroform
COSY	Correlation Spectroscopy
cm	Centimeter
J	Coupling constant in Hertz
^{o}C	Degree in Celcius
$CDCl_3$	Deuterated Chloroform
CD_3OD	Deuterated Methanol
d	Doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
EIMS	Electron Emission Mass Spectroscopy
EtOAc	Ethyl Acetate
γ	Gamma
GC	Gas Chromatography
GC-MS	Gas Chromatography – Mass Spectrometry
g	Gram
HMBC	Heteronuclear Multiple Bond Connectivity
HMQC	Heteronuclear Multiple Quantum Correlation
Hz	Hertz
OH	Hydroxy
IC	Inhibition Concentration
IR	Infra- Red
Kg	Kilogram
Lit.	Literature
m/z	Mass per charge
MS	Mass spectrum/spectra/spectrometer/spectrometry
MeOH	Methanol
OCH_3	Methoxy
m.p.	Melting point
mL	Mililitre
mg	Miligram
µg	Microgram
M^+	Molecular ion
m	Multiplet
nm	Nanometer
NMR	Nuclear Magnetic Resonance
ppm	Parts per million
^{1}H	Proton
KBr	Potassium Bromide
q	Quartet

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s	Singlet
TLC	Thin Layer Chromatography
t	Triplet
UV	Ultra Violet
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Natural Products

Many important basic discoveries in organic chemistry were carried out in the field of organic chemistry research. Natural products refer to those organic compounds, which are found in nature and are associated with living organisms. Most natural product used for studies have usually been obtained from plants and microorganisms since the practical difficulties in extracting them from animal are much greater (Paul, 1992). Organic natural products are constructed of carbon, hydrogen and oxygen atoms; frequently nitrogen atoms are also involved, and less frequently sulphur, phosphorous, chlorine, bromine, and iodine atoms (Tedder *et al.,* 1972). These compounds are divided into two classes; primary and secondary metabolites. Natural products researchers are more interested in secondary metabolite compounds that are formed from the process which does not show the cell functions clearly such as alkaloids, steroids, terpenoids, phenolic compounds, glycosides and others. This is due to the existence of many outstanding compounds from secondary metabolites which display interesting biological activities.

Natural product chemistry has lately undergone explosive growth in isolation techniques, synthetic methods, physic-chemical measurements, and new concept. On the other hand, it is precisely the chemistry of the natural product which has fostered many of the new developments because of the variety of compound available (Nakanishi *et al.,* 1974).

In recent years, modern strategies have been employed in which bioassay-guided (mainly *in vitro*) isolation and identification of active "lead" compounds from natural sources was employed, besides production of natural products libraries, production of active compounds in cell or tissue cultures, genetic manipulation, natural combinatorial chemistry and others. Natural product research is now more focused on bioactivity and the concepts of dereplication, chemical fingerprinting, and metabolomics have been introduced. In addition, selection of organisms also included those randomly selected (Sarker *et al*., 2006). In general, the plant extract contains low concentration of active compounds and a large number of compounds, requiring the use of sensitive bioassay suitable for the wide chemical variety and small amount of the tested samples. Test must be simple, reproducible, fast and cheap (Souza and krish, 1996).

1.2 Medicinal plant

The study of natural products continuous to be a major force in the development of the field of organic chemistry and medicinal chemistry. Higher plants used in traditional medicine provided some of the first prototype drugs used clinically in the treatment of a

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wide variety of diseases. The need to purify natural products from complex mixtures and to determine the structures has driven the development of more sophisticated methods for separation of compound and for their structural analysis by chemical and subsequently, spectroscopy means. Medicinal plants provide a cost-effective means of primary health care to millions of people around the world. The demand for medicinal plants is steadily increasing in both developing and developed countries due to the growing recognition of drugs based on natural product, food supplements and flavours. Being non-narcotic, having less side effects and easy availability at affordable prices makes these products sometimes the only source of health care available to the poor (Ramawat and Merillon, 2008).

Plant drugs (also called phytomedicines of phytopharmaceuticals) are plant-derived medicines that contain a chemical compound or more usually mixtures of chemical compound that act individually or in combination on the human body to prevent disorders and to restore or maintain health. Chemical entities are pure chemical compounds (isolated from natural sources such as plants, or produced by chemical synthesis) that are use for medicinal purposes (usually with a clearly defined and tested mode of action).

1.3 Zingiberaceae

Zingiberaceae is among the plant families which are widely distributed throughout the tropics particularly in Southeast Asia. Zingiberaceae is one of the largest plant families from the order Zingiberales, with approximately 50 genera and over 1,000 species. In Peninsular Malaysia, the Zingiberaceae are a component of the herbaceous ground flora of the rainforest. It is estimated that there are 150 species of ginger belonging to 23 genera found in Peninsular Malaysia. Zingiberaceae species grow naturally in damp, shaded parts of the low-land or on hill slopes, as scattered plants of thickets. Most members of the family are easily recognized by the characteristics aromatic leaves and fleshy rhizome when both of them are crushed and also by elliptic to elliptic-oblong leaves arranged in two ranks on the leaf-shoot (Holtum, 1950).
In the Southeast Asia region, several species of zingiberaceae are used as spices, medicines, flavouring agents and as source of certain dyes. Several species from the genera *Boesenbergia*, *Curcuma*, *Alpinia*, *Amomum*, *Costus*, *Kaempferia* and *Zingiber* are major ingredients in traditionally prepared tonics, locally called ‘jamu’ which are commercially available. Various ginger rhizobia provide health-promoting effects and have been utilized to treat certain illnesses such as nausea, motion sickness, stomachic, asthma, diarrhea, digestive, disorder, vomiting, rheumatism, swelling, common cold, cough and other disorder (Habsah *et al*., 2000).

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1.3.1 *Curcuma*

The genus *Curcuma* (Figure 1.1) belongs to the tribe Zingibereae and consists of about 80 species of rhizomatous herbs. It is native to the warm and humid environments. It has widespread adaptation from the sea level to altitude as high as 2000 m in the Western Ghats and Himalayas. Species such as *C. longa*, *C. angustifolia*, *C. neilgberrensis*, *C. kudagensis*, *C. pseudomontana* and *C. coriacea* are confined to hills at 1000-2500 metre altitude. It is considered to have originated in the Indo-Malayan Region and widely distributed in the tropics of Asia to Africa and Australia. About 40 out of the 100 or so species reported in the Genus are of Indian origin (Sasikumar, 2005).

1.3.2 *Curcuma mangga* Valeton and Van Zijp

Curcuma mangga is locally known as "mango ginger" or "manggo turmeric" while in Indonesia, it is recognized as *tĕmu mangga* (general), *tĕmu poh*, *tĕmu banjangan, tĕmu lalab*, *konèng lalab* (Sundanese), *tĕmu pauh* (Java), *temo pao* (Madurese), *konèng joho* and *konèng parè* (Burkill, 1966; Abas *et al*., 2005 and Bos *et al*., 2007). This plant commonly grown in Thailand, Peninsular Malaysia, Bengal, North, Eastern India and Java**.** The rhizomes are very similar to ginger but have a raw mango smell when the fresh rhizomes are cut. So it becomes popular vegetable which the tips of young rhizomes and shoots are consumed raw with rice and also used in making pickles in south India (Abas *et al*., 2005 and Liu and Nair, 2010). Medicinally, the rhizomes are used as a stomachic and for chest pains, fever, gastric ulcer and general debility. It is also used in postpartum care, specifically to aid womb healing (Abas *et al*., 2005 and Ruangsang *et al*., 2010).

1.4 *Boesenbergia*

Boesenbergia belongs to ginger family, Zingiberaceae in the order of Zingiberales. The genus of about 80 species is distributed from India to South East Asia. Borneo, is one of the two distribution centres apart from Thailand, which is estimated to have 25 species (Larsen *et al*., 2003). *Boesenbergia* species is extremely rare compare to other genera. Mostly, they are found in very damp, shaded areas and are usually close to streams or in boggy conditions. Thus, there is an urgency to document the plants before facing extinction. Many researchers have shown that the rhizome of *Boesenbergia* displayed health-benefits properties. For instance, the rhizome of *B. rotunda* is generally used as a culinary spice in Thailand and also has been used for the treatment of oral diseases (that is dry mouth), stomach discomfort, stomach pain, leucorrhoea, diuretic, dysentery, and inflammation. The rhizomes are used in traditional medicine as antiseptic and for the treatment of stomach ache (Hasnah *et al*., 1995), diarrhea, dermatitis, dry cough and mouth ulcers (Burkill, 1935 and Heyne, 1987), gastrointestinal disorders and post-natal treatment (Burkill, 1935).

1.4.1 *Boesenbergia prainiana* (King ex Baker) Schltr.

Boesenbergia prainiana (Figure 1.2) is grown in peninsular Malaysia and it is distributed in the lowland forest in the states of Terengganu, Perak, Pahang and Johor. It is a few-leaved herb up to 30 cm tall and have inflorescence terminal on leafy shoots. It flowers from the top, flower parts are delicate and short-lived.

1.5 Leguminosae

Many plants from the Leguminosae family are medicinal herbs which are easily found in Malaysia. There are mostly tropical and subtropical trees and shrubs comprising about 150 genera and 2,200 species. The leaves are alternate but may be bipinnate or simple. They are grown as weeds (i.e. *Mimosa pudica*), woody shrubs (*Peltophorum pterocarpum*), crops (*Arachis hypogaea*) and vines (*Bauhinia kockiana*). Some of these plants are edible, and hence they are utilised for various purposes in food, beverages, and food colouring agent (Goh, 2004 and Ong, 2006). These plants are commonly used as traditional medicines to treat various health complications

1.5.1 *Bauhinia*

The genus *Bauhinia* belongs to the Leguminosae family, sub-family Cesalpiniaceae and comprises about 300 species distributed in tropical and subtropical regions. *B. kockiana*, a tropical vine is cultivated as a garden ornamental plant because of its bright orange–red magnificent inflorescences. This plant originates from the Malaysia tropical forest and its roots are used by the Kelabit ethnic group in Sarawak to treat gonorrhoea (Fasihuddin *et al*., 1995). Besides, the infusion of the roots is consumed orally to treat nervous debility, insomnia and fatigue. The bark and root are also used traditionally to treat toothache (Ong, 2006).

1.5.2 *Bauhinia thonningii* Schum.

Bauhinia thonningii (Figure 1.3) is a savanna deciduous plant which has a wide distribution range in tropical Africa and extending from West Africa to the Sudan and south wards to east and central southern Africa, including countries such as Mozambique, Malawi, Zimbabwe, Zambia, Botswana, Tanzania and Namibia. *B. thonningii* trees typically grow to a height of 6 to 12 m and their branches spread 3 to 6 m outwards. The flowers are usually five-petaled and are 7 to 12 cm in diameter, generally in shades of red, pink, purple, orange or yellow colour. The species is widely used in sub-Saharan Africa for poles, firewood, charcoal and its pods are eaten by wild animals. The plant is also used to make ropes, dyes and gums and are used in ethnomedicine (Chidumayo, 2007 and Chidumayo, 2008).

1.6 Problem Statements

Plants with ethobotanical or ethnopharmaceutical activities are acceptable as healthy-giving supplements. Plants have a long history of use in the treatment of cancer but many claims for the efficacy of such treatment. These have prompted us to carry out investigation of the three medicinal plant species which are *Curcuma mangga* Valeton and Van Zijp., B*oesenbergia prainiana* (King ex Baker) Schltr. and *Bauhinia thonnigii* Schumach. & Thonn. These plants have long been utilized as traditional medicine either consume internally or apply externally and also consumed as food or vegetable.So further investigation been carry out in term of phytochemistry and bioactivity studies. For *curcuma mangga*, there were not much previous research on the chemical constituents and biological activity studies while for *Boesenbergia prainiana* and *Bauhinia thonningii* there were no phytochemical and biological activity studies previously.

1.7 Objectives of Research

Objectives of the research are:

1. To extract and isolate the chemical constituents of *Curcuma mangga* Valeton and Van Zijp., *Boesenbergia prainiana* (King ex Baker) Schltr. and *Bauhinia thonnigii* Schumach. & Thonn.

2. To elucidate the structures of isolated compounds using various spectroscopic methods such as IR, MS and NMR.

3. To screen the bioactivities of the crude extracts and isolated compounds through various bioactivy studies such as cytotoxic, antioxidant, total phenolic content, antimicrobial and antifungal.

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