

THE EXTRACTION OF *Clinacanthus Nutans* WITH THREE
SOLVENTS USING THE MIXTURE DESIGN, AND ITS EXTRACTS
ANTICANCER ACTIVITY

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AUTHOR DECLARATION

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I hereby declare that the work in this dissertation is my own except for quotations and summaries which have been duly acknowledged.

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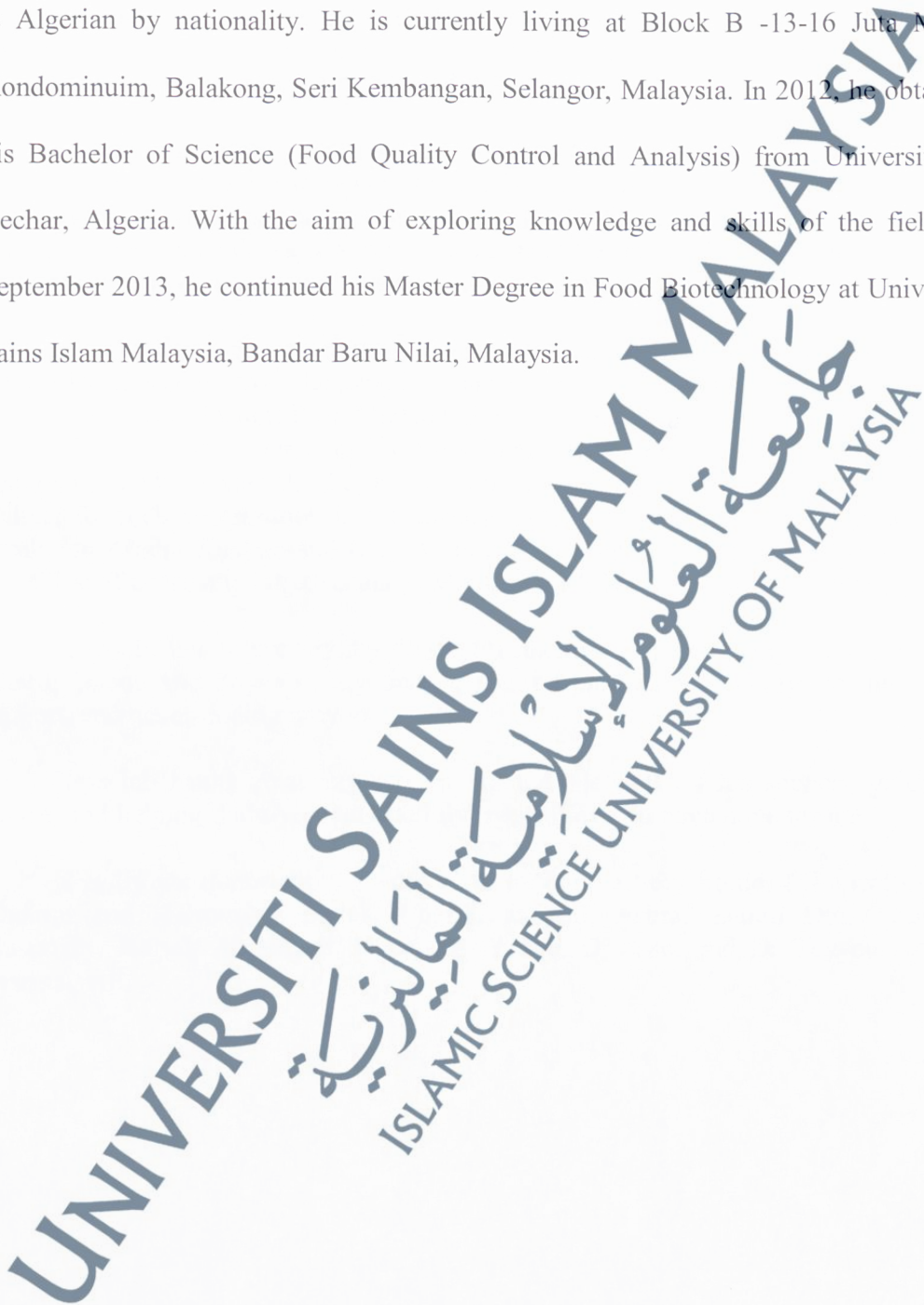
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ABSTRAK

Clinacanthus nutans (Acanthaceae) Juga dikenali sebagai Belalai Gajah di Malaysia. Tumbuhan ini merupakan salah satu ubatan herba yang penting yang pada kebiasaannya tumbuh di kawasan Asia Tropika. Tumbuhan ini digunakan secara tradisional sebagai anti-keradangan, anti-hepatitis dan anti-herpes. Kajian terbaru menunjukkan tumbuhan ini mempunyai nilai perubatan yang tinggi untuk rawatan kanser. Dalam kajian ini, pengekstrakan menggunakan pelarut yang berbeza (kloroform, metanol, dan heksana) dan kombinasi ketiga-tiga pelarut ini di dalam rekabentuk tiga campuran digunakan untuk mengekstrak kompaun biokimia dalam *Clinacanthus nutans* kompaun. Kompaun ini dikenalpasti menggunakan kromatografi gas- spektrometri jisim GC-MS. Potensi kesan antikanser dalam ekstrak *Clinacanthus nutans* pada sel 38 kanser usus besar sel menggunakan asai MTT juga telah dinilai dan nilai IC₅₀ didapati pada 6µg/ml dari campuran tiga pelarut. Keputusan GC-MS menunjukkan pengekstrakan menggunakan kombinasi kloroform dan heksana dapat mengekstrak kompaun yang paling banyak diikuti oleh heksana seterusnya ialah kombinasi ketiga-tiga pelarut. Manakala kombinasi ekstrak yang lain memperoleh kompaun yang kurang dibandingkan dengan gabungan pelarut yang dinyatakan sebelum ini. Pengekstrakan daripada gabungan ketiga tiga pelarut menunjukkan potensi yang lebih baik dalam menghalang pertumbuhan sel kanser, dengan nilai IC₅₀ 6 µg/ml diikuti dengan kloroform seterusnya oleh kombinasi ekstrak kloroform dan heksana. Ekstrak yang lain tidak mampu menghalangnya melebihi daripada 50% pertumbuhan sel.

Kata kunci: *Clinacanthus nutans*, Balalai Gajah, rakabentuk tiga campuran, GC-MS, antikanser dan MTT assay.

ABSTRACT

Clinacanthus nutans (Acanthaceae), known in Malaysia as “Belalai Gajah” is one of the important herbal medicines grown in tropical part of Asia. It has been traditionally used as anti-inflammation, anti-hepatitis and anti-herpes agent. Recent studies have also shown its high medicinal values for the treatment of cancer. Here in this study, the extraction using different solvents (chloroform, methanol and hexane) and their combinations have been used to extract biochemical compounds in *Clinacanthus nutans*. The compounds have been determined using gas chromatography–mass spectrometry (GS-MS). The potential anticancer effect of *Clinacanthus nutans* extracts on colon 38 cancer cells using MTT assays have also been evaluated and the IC₅₀. The result of GC-MS showed that the extraction using the combination of chloroform and hexane extracted the maximum number of compounds followed by hexane and combination of three solvents. While the other combinations extracted less number of compounds as compared with above combinations. The extract of three solvents mixtures exhibited a better growth inhibition with IC₅₀ 6 µg/ml followed by chloroform extract 15 µg/ml and the combination of chloroform and hexane extract with 19 µg/ml. Other extracts were not able to inhibit up to 50 % of the cell growth. This study suggests that phytochemical constituents present in *Clinacanthus nutans* extract could be as an alternate drug for anti-cancer. However, additional research needs to be done to understand the mechanism of its anticancer compounds.

Keywords:

Clinacanthus nutans, Belalai Gajah, three mixture design, GC-MS, anticancer activity, MTT assay.

ملخص البحث

نبته (*Clinacanthus nutans* (Acanthaceae) او كما يطلق عليها في ماليزيا Belalai Gajah احدى اهم النباتات الطبية التي تنمو بالاقليم الاستوائية في قارة اسيا. استعملت قديما كمضاد للالتهاب , مضادة لالتهاب الكبد الوبائي ومضادة الحلا (الهربس). الدراسات الحديثة اثبتت ان لهذه النبتة قيمة طبية عالية لمعالجة السرطان. في هذا المشروع , نقوم بعملية الاستخلاص مستخدمين في ذلك انواع مختلفة من المحاليل (الكلوروفورم, الميثانول و الهكسان) بحيث تتم عملية الاستخلاص باستخدام كل محلول على انفراد ثم نقوم بمزج كل محلولين مع بعضهما ومن ثم نقوم بمزج المحاليل مع بعضها البعض وذلك لرؤية مدى فعالية كل محلول في استخلاص اكبر قدر ممكن من المركبات البيوكيميائية. يتم استعمال تقنية الفصل الكروماتوغرافي بالغاز (GC-MS) وذلك للتعرف على المركبات التي تم استخلاصها. يتم مقدرة نبته *Clinacanthus nutans* على مقاومة السرطان تتم تجربتها على خلايا القولون المسرطنة باستعمال تقنية MTT. اظهرت نتائج الفصل الكروماتوغرافي ان مزيج الكلوروفورم مع الهكسان اعطى اكبر قدر ممكن من المركبات يليه مستخلص الهكسان منفردا ويليه مستخلص مزيج المركبات الثلاث مع بعض. بينما اظهرت باقي المستخلصات عدد مركبات اقل مقارنة بسابقتها. بالنسبة لتقنية MTT فان مستخلص المحاليل الثلاث مع بعض ادى الى تثبيط نمو الخلايا احسن من باقي المستخلصات يليه مستخلص الكلوروفورم منفردا ويليه مستخلص مزيج الكلوروفورم مع الهكسان . باقي المستخلصات لم تكن لها القدرة على تثبيط نمو الخلايا من 50 % من نمو الخلايا.

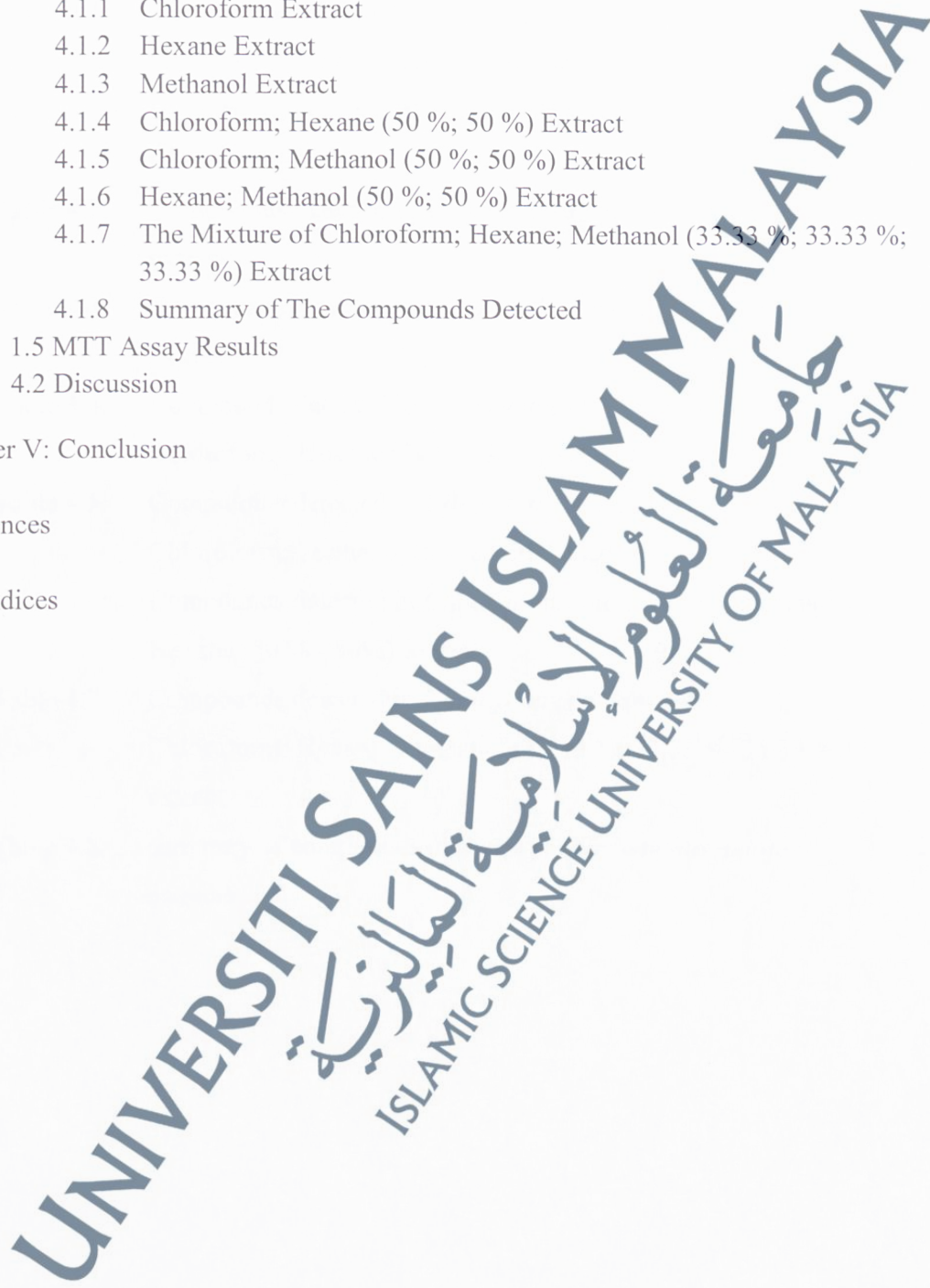
الكلمات المفتاحية:

Clinacanthus nutans, Belalai Gajah, مزج المحاليل, GC-MS, الفصل المضاد للسرطان, MTT assay.

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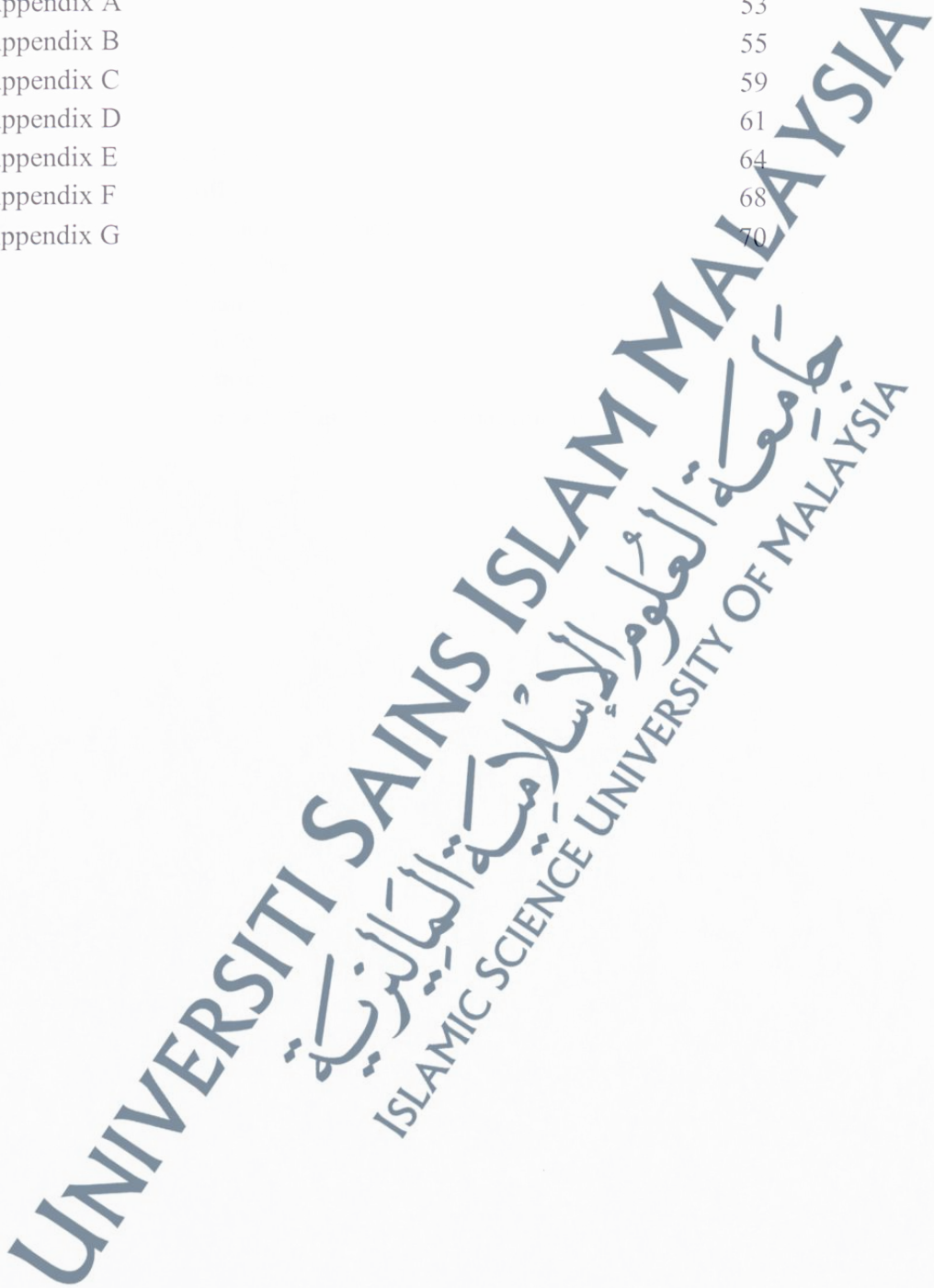
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ABBREVIATION

GC-MS	Gas Chromatography - Mass spectroscopy
cm	Centimeter
mm	Millimeter
mL	milliliter
g	gram
°C	Celsius
mg	milligram
rpm	Rotation per minute
μL	microliter
min	minute
Da	Dalton
μg	microgram
IC ₅₀	Concentration of extract that inhibits cell growth by 50 %.

