



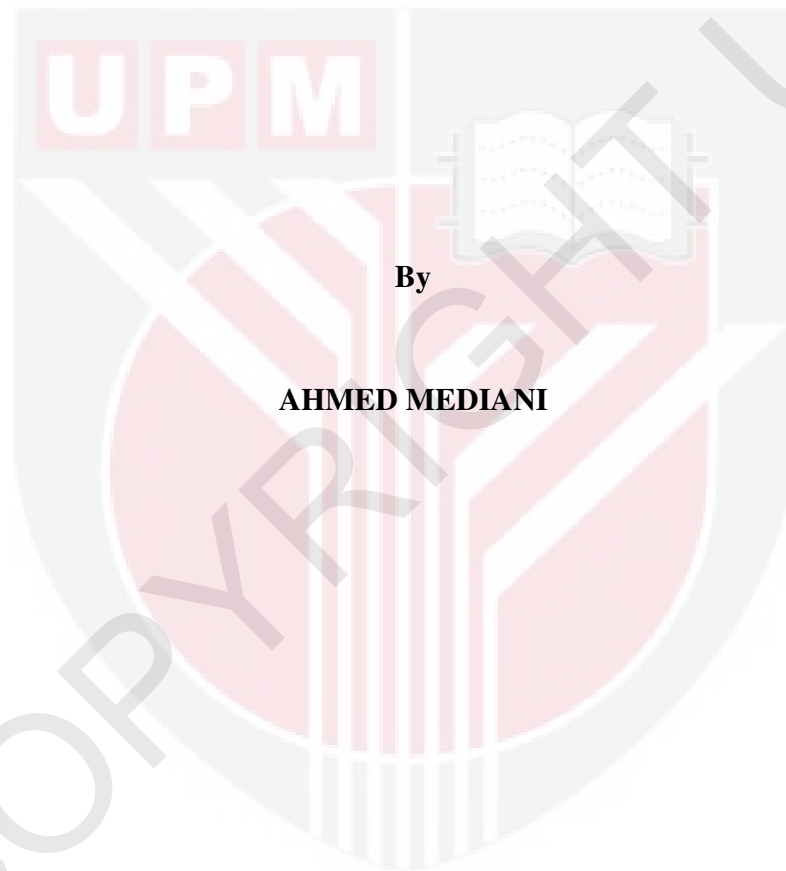
UNIVERSITI PUTRA MALAYSIA

***INFLUENCE OF GROWTH STAGES AND HARVESTING SEASON, AND
DRYING METHODS ON PHYTOCHEMICAL CONTENT AND ANTIOXIDANT
ACTIVITY OF *Cosmos caudatus* KUNTH LEAVES***

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By

AHMED MEDIANI

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

May 2012

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

INFLUENCE OF GROWTH STAGES AND HARVESTING SEASON, AND DRYING METHODS ON PHYTOCHEMICAL CONTENT AND ANTIOXIDANT ACTIVITY OF *Cosmos caudatus* KUNTH LEAVES

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May 2012

Chairman: Associate Prof. Faridah Abas, PhD

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Cosmos caudatus which is locally known as ‘ulam raja’, is one of the local Malaysian herbs used traditionally as medicinal herbal in treating several maladies and as a food because of the general confidence of its high antioxidant activity (AA). The main aim of this study was to determine the preferable drying methods, suitable harvesting plant age and best growing season for obtaining valuable *C. caudatus* products. Optimizing oven drying conditions (e.g. drying time and oven temperature) has been shown to affect AA and total phenolic content (TPC) of this particular herb when the response surface methodology was applied with central composite design (RSM CCD). The assessments of various growth stages (8, 10, and 12 weeks) and season on TPC and AA were also conducted. Meanwhile, the differences among oven-, air- and freeze-dried *C. caudatus* were determined, and the dried samples were compared with the frozen samples (FS) to evaluate the effects of storage time on its TPC and AA. ¹H nuclear magnetic resonance

(¹H-NMR) combined with principal components analysis (PCA) and partial least-squares discriminate analysis (PLS-DA) was applied to distinguish variations of *C. caudatus* samples processed with various drying techniques.

Results showed that the optimized conditions of oven drying (OD) method for 80% methanol and 80% ethanol extract were 44.5°C for 4 h and 43.12°C for 4.05 h, with IC₅₀ of 0.045 and 0.055 mg/ml, respectively. The predicted values for TPC in optimized conditions for 80 % methanol and 80% ethanol were 16.5 and 15.8 mg GAE/100 g DW, respectively. Eight-week-old herbs harvested at dry season exhibited the highest TPC and the lowest IC₅₀ values. The difference in TPC was shown to be significant ($P \leq 0.05$) among the drying methods and the three development stages, while AA exhibited considerable changes for the three growth stages studied. Except for oven drying, the difference between freeze- and air- drying was rather insignificant. After 120 days of storage, the dried samples showed a high and consistent AA compared to the frozen material.

As for the metabolomics study, PCA and PLS scores revealed noticeable clear separated clusters representing the three drying methods by PC1 and PC2, with an eigenvalue of 77.9%. Various assigned signals that were referring to metabolites responsible for the sample variation were also ascribed. The identified compounds were β glucose, chlorogenic acid, rutin, quercetin, quercetin 3-*O*-rhamnoside, quercetin 3-*O*- β -arabinofuranoside and quercetin 3-*O*- β -glucoside. The identification of metabolites was further confirmed through LC-MS/MS profiling and by comparing with NMR spectra of

pure compounds. The findings obtained in this study showed that *Cosmos caudatus* could be used as a potential source of antioxidant, and a versatile herb for medicinal applications.



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

**PENGARUH PERINGKAT PERTUMBUHAN, MUSIM MENUAI DAN KAEDAH
PENGERINGAN PADA KANDUNGAN FITOKIMIA DAN AKTIVITI
ANTIOKSIDAN DAUN *C. CAUDATUS***

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Cosmos caudatus atau 'ulam raja' adalah salah satu herba tempatan Malaysia yang digunakan untuk tujuan perubatan bagi merawat beberapa penyakit dan juga sebagai makanan yang tinggi aktiviti antioksidan (AA). Tujuan utama kajian ini adalah untuk menentukan pengaruh kesan pemprosesan ke atas manfaat dan keberkesanan *C. caudatus*. Pengeringan ketuhar yang optimum (masa pengeringan dan suhu ketuhar) mempengaruhi perencatan radikal bebas dan jumlah kandungan fenolik (TPC) dalam herba ini yang telah dijalankan menggunakan aplikasi Metodologi Tindakbalas Permukaan dengan Rekaan Komposit Tengah (RSM CCD). Perbandingan antara pengeringan ketuhar, udara dan sejuk beku telah ditentukan dan sampel yang kering dibandingkan dengan sampel beku (FS) untuk menilai kesan penyimpanan. Penilaian terhadap pelbagai peringkat usia pertumbuhan (8,10 dan 12 minggu) dan kesan musim terhadap TPC dan IC₅₀ juga telah

dijalankan. Pengeringan *C. caudatus* secara udara, sejuk beku dan ketuhar juga telah dibezakan oleh metabolomik berasaskan $^1\text{H-NMR}$ dan analisis multivariat untuk menilai perubahan metabolit selepas menggunakan kaedah-kaedah ini.

Keputusan menunjukkan bahawa syarat optimum untuk kaedah pengeringan ketuhar bagi 80% methanol dan 80% etanol adalah 44.5°C untuk 4 jam dan 43.12°C untuk 4.05 jam dengan masing-masing 0.045 and 0.055 mg/ml IC_{50} . Nilai-nilai yang dianggarkan bagi TPC pada syarat yang optimum untuk 80% methanol dan 80% etanol adalah masing-masing 16.5 dan 15.8 mg GAE/100 g DW. Perbezaan TPC adalah bererti pada $P < 0.05$ antara kaedah-kaedah pengeringan dan tiga peringkat pertumbuhan manakala AA menunjukkan perbezaan bererti antara ketiga-tiga peringkat pertumbuhan dan perbezaan tidak bererti antara kaedah pengeringan sejuk beku dan udara, kecuali bagi pengeringan ketuhar. Selepas minggu ke-8, herba yang dituai pada musim kemarau ini menunjukkan nilai TPC yang tertinggi dan nilai IC_{50} yang terendah. Selepas 120 hari penyimpanan, sampel yang kering menunjukkan AA yang tinggi dan konsisten apabila dibandingkan dengan sampel beku.

Bagi kajian metabolomik, nilai PCA dan PLS menunjukkan pengasingan kelompok yang jelas yang mewakili tiga kaedah pengeringan oleh PC1 dan PC2 iaitu 77.9%. Pelbagai petunjuk yang merujuk kepada fungsi metabolit untuk variasi sampel telah ditentukan. Sebatian yang telah dikenalpasti adalah β glukosa, asid klorogenik, rutin, kuersetin, kuersetin 3-*O*-ramnosida, kuersetin 3-*O*- β -arabinofuranosida and kuersetin 3-*O*- β -glukosida. Pengenalpastian metabolit telah disahkan menggunakan profil LC-MS/MS dan

dibandingkan dengan sebatian tulen spektrum NMR. Hasil yang diperolehi dari kajian ini menunjukkan bahawa *C. caudatus* boleh digunakan sebagai sumber yang berpotensi dari segi antioksidan dan juga herba yang serba guna dalam aplikasi perubatan.



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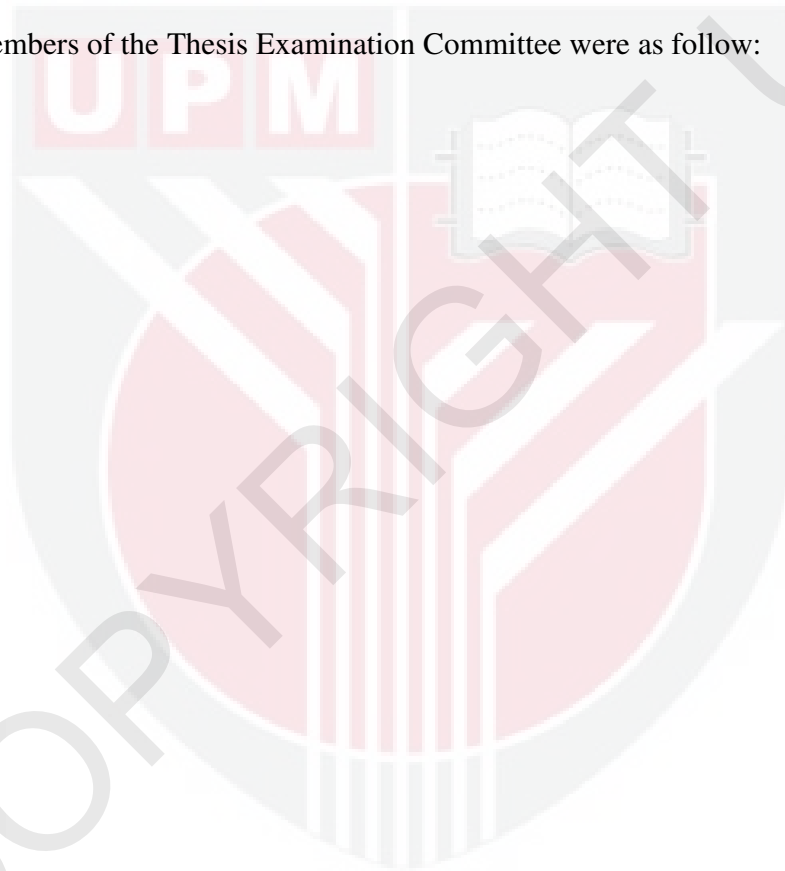
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I certify that a thesis examination committee has met on..... 2012 to conduct the final examination of Ahmed Mediani on his thesis entitled ‘‘Influence of Drying Methods, Growth Stages and Harvesting Season on Phytochemicals Content and Antioxidant Activity of *Cosmos Caudatus* Leaves□□ in accordance with the Universities College Act 1971 and the constitution of the Unversiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science. Members of the Thesis Examination Committee were as follow:



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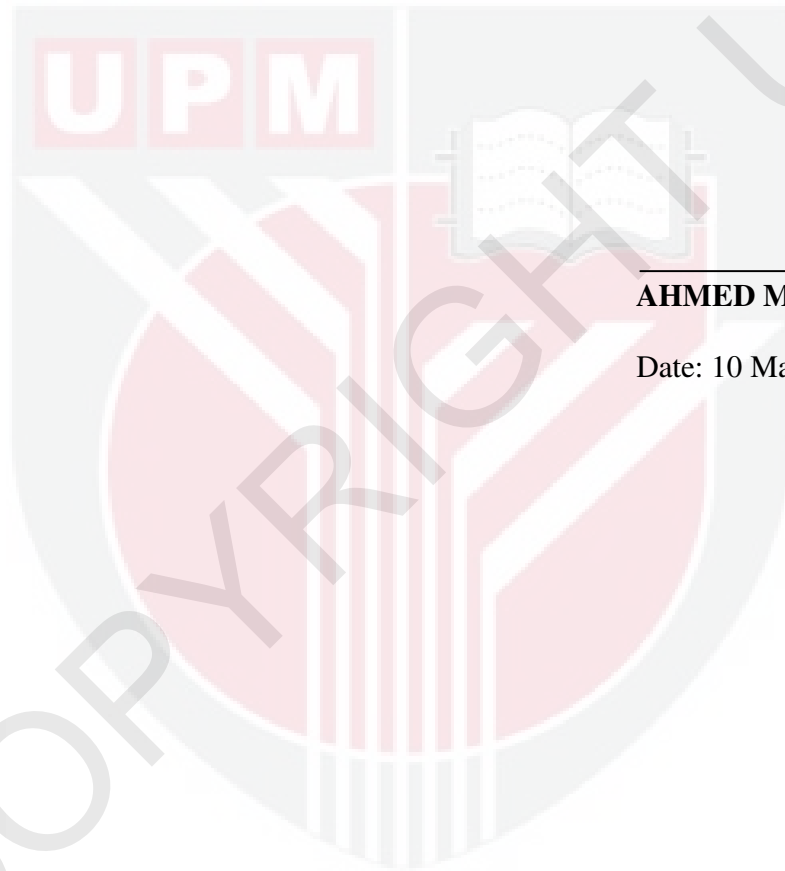
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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



AHMED MEDIANI

Date: 10 Mai 2012

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