PHYTOCHEMICALS AND BIOACTIVITIES OF *Cinnamomum porrectum* (ROXB.) KOSTERM AND *Cinnamomum mollissimum* HOOK F.

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Dedicated to:

My father, Masnon bin Ab Rahim
My mother, Masitah binti Md Tab
My brothers and my sister
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PREFACE

This thesis is the result of my work carried out in the Department of Chemistry, Universiti Teknologi Malaysia between September 2011 and September 2013 under the supervision of Assoc Prof. Dr. Farediah Ahmad. Parts of my works described in this thesis have been reported in the following publications:


3. Fatin Fasihah Masnon and Farediah Ahmad. Phytochemicals and Bioactivities of *Cinnamomum mollissimum*. Poster presented at the International Conference on Natural Products 2013 (ICNP 2013) at Shah Alam Convention Centre (SACC), Selangor, 4-6 March 2013.
ABSTRACT

*Cinnamomum porrectum* (Roxb.) Kosterm and *Cinnamomum mollissimum* Hook f. which belong to the Lauraceae family are widely distributed in Peninsular Malaysia. They are locally known as “medang kemangi” and “medang lawang”, respectively. The leaves and barks of *C. porrectum* and the leaves of *C. mollissimum* were extracted by cold extraction using methanol and the extracts were then partitioned using different solvents with increasing polarity to yield the petroleum ether, chloroform and ethyl acetate extracts. Acidification, basification and extraction of the methanol extract from the barks of *C. mollissimum* with chloroform produced the neutral and alkaloid crude extracts. The isolation and purification on the crude extracts were achieved using chromatographic techniques and have resulted in the isolation of prenylpropanoid, triterpenes, ester, carboxylic acid and aporphine alkaloids. Structure of the isolated compounds were elucidated using spectroscopic techniques including infrared, ultraviolet-visible, nuclear magnetic resonance spectroscopies, mass spectrometry and also by comparison of the spectral data with those previously reported in the literatures. Purification process of the leaves extracts of *C. porrectum* have yielded three compounds identified as methyl eugenol, β-sitosterol and stigmast-4-en-3-one. Benzyl benzoate and benzoic acid have been isolated from the leaves of *C. mollissimum*. Purification of the alkaloid extract from the barks of *C. mollissimum* produced five aporphines, namely isocorydine, N-methylhernagine, N-methylhernovine, hernagine and hernovine. Several bioactivities such as antibacterial, antioxidant and antityrosinase have been investigated for the crude extracts and selected compounds. The antibacterial assays were performed using disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The results showed that the alkaloid extract, methyl eugenol and benzyl benzoate exhibited strong antibacterial activity towards selective bacterial strains with the concentration ranged less than 500 μg/mL. The antioxidant activity by DPPH showed significance results on the alkaloid extract and hernovine with SC₅₀ 50.1 μg/mL and 50 μg/mL, respectively. The crude extracts which were screened for antityrosinase activity using mushroom tyrosinase were found to be inactive with IC₅₀ > 1000 μg/mL. As a conclusion, the alkaloid extract showed good activity towards all the tested bioassays except for the tyrosinase inhibition assay. The activity portrayed was due to the synergistic effect between the compounds presence in the extract.
**ABSTRAK**

Spesies *Cinnamomum porrectum* (Roxb.) Kosterm dan *Cinnamomum mollissimus* Hook f. tergolong dalam keluarga Lauraceae ditemui dengan meluas di Semenanjung Malaysia. Nama tempatan untuk masing-masing spesies ini adalah medang kemangi dan medang lawang. Daun dan batang kering *C. porrectum* dan *C. mollissimum* telah diekstrak menggunakan teknik rendaman dengan pelarut metanol dan kemudiannya ekstrak tersebut diperingkatkan menggunakan pelarut yang berbeza kekutuban untuk menghasilkan ekstrak petroleum eter, kloroform dan etil asetat. Pengasidan, pembesan dan pengekstrakkan ekstrak metanol daripada batang *C. mollissimum* menggunakan kloroform telah menghasilkan ekstrak neutral dan ekstrak alkaloid. Pengasahan dan penulenan setiap ekstrak mentah dijalankan dengan menggunakan teknik kromatografi dan telah berjaya menghasilkan sebatian fenilpropanoid, triterpena, ester, asid karboksilik dan alkaloid. Pengenalpastian struktur kimia sebatian tulen dilakukan dengan menggunakan spektroskopi inframerah, ultralembayung-nampak, resonans magnet nukleus, spektrometri jisim, dan juga perbandingan data spektrum dengan data yang telah diterbitkan dalam literatur. Proses penulenan terhadap ekstrak mentah daripada daun *C. porrectum* telah menghasilkan sebatian kimia yang dikenalpasti sebagai metil eugenol, β-sitosterol dan stigmast-4-en-3-on. Benzil benzoat dan asid benzoik telah berjaya dipisahkan daripada ekstrak daun *C. mollissimum*. Penulenan terhadap ekstrak alkaloid daripada batang *C. mollissimum* telah menghasilkan lima alkaloid dinamakan sebagai isokoridin, *N*-metilhernagin, *N*-metilhernovin, hernagin dan hernovin. Beberapa bioaktiviti seperti antibakteria, antioksidan dan antitirosinase telah dikaji ke atas setiap ekstrak mentah dan sebatian terpilih. Saringan antibakteria yang telah diulang menggunakan kepekatan rencatan minimum (MIC) dan kepekatan bakterisida minimum (MBC). Keputusan telah menunjukkan bahawa ekstrak alkaloid, metil eugenol dan benzil benzoat mempunyai aktiviti antibakteria yang kuat terhadap beberapa jenis bakteria terpilih dalam jual kepekatan kurang daripada 500 μg/mL. Aktiviti antioksidan dengan menggunakan DPPH menunjukkan keputusan yang signifikan ke atas ekstrak alkaloid dan hernovin masing-masing dengan SC_{50} 50.1 μg/mL dan 50 μg/mL. Semua ekstrak mentah yang disaring untuk aktiviti tirosinase menggunakan tirosinase cendawan didapati tidak aktif dengan IC_{50} > 1000 μg/mL. Kesimpulannya, ekstrak alkaloid mempunyai aktiviti yang baik terhadap kecekupan ujian bioaktiviti kecuali saringan perencatan tirosinase. Aktiviti yang dipamerkan disebabkan oleh kesan sinergi yang berlaku antara sebatian yang terdapat di dalam ekstrak tersebut.