

**PHYTOCHEMICAL CONSTITUENTS,
ANTIOXIDANT AND CHEMOPREVENTIVE
EFFECTS OF SELECTED PLANTS AGAINST
CARBON TETRACHLORIDE (CCL₄)- INDUCED
HEPATIC DAMAGE IN RATS**



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UMS
UNIVERSITI MALAYSIA SABAH

**BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
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**THIS IS SUBMITTED IN THE FULL FILM FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY**

**BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2016**

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DECLARATION

I hereby declare that the material in this thesis is of my own effort except for the quotations, excerpts, equations, references and summaries which have been duly acknowledged and cited clearly it sources.

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ABSTRACT

Commelina nudiflora, *Nephrolepis biserrata* and *Dillenia suffruticosa* have been used in folk medicine for protection against different diseases such as jaundice, although their actual efficiency remains uncertain. The current experiment was designed to investigate the bioactive compounds and hepatoprotective potential of the selected plants against carbon tetrachloride (CCl₄)-induced hepatic oxidative damage in rats and their possible mechanism of action. The total phenolic and flavonoid contents, phytochemical constituents and antioxidant activities were determined in the methanolic extracts and methanolic fractions of plant samples. Phytochemical analysis was performed by gas chromatography and mass spectrometry (GCMS). In this *in vivo* study, Sprague Dawley rats were pre-treated with various doses of the methanolic extracts of the selected plants once daily for 14 days followed by two doses of CCl₄ (1 ml/kg b.wt.). After 24 hours of the last dosage, the rats were sacrificed and hepatoprotective analyses were performed on the rat's liver. The obtained data revealed that the methanolic extracts and methanolic fractions of *C. nudiflora*, *N. biserrata* and *D. suffruticosa* possessed strong antioxidant activities and have the ability to scavenge DPPH free radicals effectively. The GCMS analysis of the methanolic extracts and methanolic fractions of *C. nudiflora*, *N. biserrata* and *D. suffruticosa* demonstrated the presences of different bioactive compounds of various natures. The identified bioactive compounds have been reported with antioxidant, anti-inflammatory and hepatoprotective properties. The animal studies indicated that the methanolic extracts of *C. nudiflora*, *N. biserrata* and *D. suffruticosa* showed hepatoprotective effects against CCl₄-induced hepatic damage. The administration of Methanolic extracts of the plants depleted the elevation of enzymatic levels of serum transaminases, reduced the extent of malondialdehyde production, elevated the level of reduced glutathione and also increased the activities of antioxidant enzymes. The histopathological examination by light and electron microscopy indicated that these plant extracts protect the liver from the toxic effects of CCl₄ and healed lesions such as necrosis, fatty degeneration and hepatocyte injuries such as irregular lamellar organization and dilations in endoplasmic reticulum. The immunohistochemical studies revealed that the administration of plant extracts decreased the formation of 4-hydroxyl-2-nonenal (HNE) modified protein adducts and 8-hydroxy-2-deoxyguanosine (8-OHdG) as compared to the CCl₄-intoxicated group. Furthermore, the over-expression of the pro-inflammatory markers TNF- α , IL-6 and PGE2 are also reduced. Collectively, these plants alleviated CCl₄-induced hepatic damage via modulation of hepatic metabolizing enzymes, suppression of oxidative stress, inhibition of inflammatory cytokines and PGE2 in liver. These findings exhibit the potential prospects of *C. nudiflora*, *N. biserrata* and *D. suffruticosa* as functional ingredients to prevent ROS-related liver damage.

ABSTRAK

PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT AND CHEMOPREVENTIVE EFFECTS OF SELECTED PLANTS AGAINST CARBON TETRACHLORIDE (CCl₄)-INDUCED HEPATIC DAMAGE IN RATS

Commelina nudiflora, *Nephrolepis biserrata* dan *Dillenia suffruticosa* telah digunakan dalam perubatan tradisional untuk perlindungan terhadap pelbagai penyakit seperti penyakit kuning, namun/tetapi keberkesannya masih tidak dapat dipastikan. Eksperimen ini direka untuk menyiasat kompaun bioaktif dan potensi melindungi hati daripada tumbuh-tumbuhan yang terpilih terhadap rangsangan oksidatif hepatik-karbon tetraklorida (CCl₄) pada tikus dan mekanisme tindakannya. Jumlah kandungan fenolik dan flavonoid, juzuk fitokimia dan aktiviti antioksidan ditentukan dalam ekstrak metanol dan pecahan metanol daripada sampel tumbuhan-tumbuhan tersebut. Analisis fitokimia telah dilakukan dengan gas kromatografi dan jisim spektrometri (GCMS). Dalam kajian *in vivo* ini, tikus Sprague Dawley telah di pra-rawat dengan pelbagai dos ekstrak metanol daripada tumbuh-tumbuhan yang terpilih sebanyak sekali sehari selama 14 hari, diikuti dengan dua dos CCl₄ (1 ml / kg berat badan). Selepas 24 jam dos yang terakhir, tikus telah dikorbankan dan analisis tahap perlindungan hati dilakukan ke atas hati tikus. Data yang diperolehi menunjukkan bahawa ekstrak metanol dan pecahan metanol *C. nudiflora*, *N. biserrata* dan *D. suffruticosa* mempunyai aktiviti antioksidan yang kuat dan mempunyai keupayaan untuk keluar mencari radikal bebas DPPH dengan berkesan. Analisis GCMS daripada ekstrak metanol dan pecahan metanol *C. nudiflora*, *N. biserrata* dan *D. suffruticosa* menunjukkan kehadiran sebatian bioaktif yang berbeza dari pelbagai sifat. Sebatian bioaktif yang dikenalpasti telah dilaporkan mempunyai kesan antioksidan, anti-radang dan perlindungan hati. Kajian haiwan menunjukkan bahawa ekstrak metanol daripada *C. nudiflora*, *N. biserrata* dan *D. suffruticosa* mempunyai kesan perlindungan hati akibat daripada rangsangan kerosakan hati-CCl₄. Pemberian ekstrak metanol daripada tumbuh-tumbuhan menurunkan tahap enzim serum transaminases hati, mengurangkan tahap pengeluaran malondialdehyde, meningkatkan tahap glutation terturun di samping meningkatkan aktiviti enzim antioksidan. Pemeriksaan histopatologi menggunakan cahaya dan mikroskop elektron menunjukkan bahawa ekstrak daripada tumbuhan ini melindungi hati daripada kesan toksik CCl₄ dan menyembuhkan luka seperti nekrosis, degenerasi lemak dan kecederaan sel hati seperti organisasi lamela yang tidak teratur dan dilatasi dalam endoplasmic reticulums. Kajian immunohistochemical mendapati bahawa pemberian ekstrak tumbuhan telah menurunkan pembentukan '4- hidroksil-2-nonenal (HNE) modified protein adducts' dan '8-hydroxy- 2- deoxyguanosine (8-OHdG)' berbanding dengan kumpulan yang telah menerima CCl₄. Tambahan pula, pembentukan yang tidak terkawal oleh penanda pro-inflammatori TNF- α , IL-6 dan PGE2 juga dapat dikurangkan. Secara kolektif, tumbuhan ini mengurangkan kerosakan hati akibat CCl₄ melalui modulasi enzim metabolisme hati, penindasan oksidatif stress, perencatan inflammatori cytokines dan PGE2 di dalam hati. Penemuan ini menunjukkan potensi prospek *C. nudiflora*, *N. biserrata* dan *D. suffruticosa* sebagai bahan berfungsi untuk mencegah kerosakan hati Berkaitan ROS.

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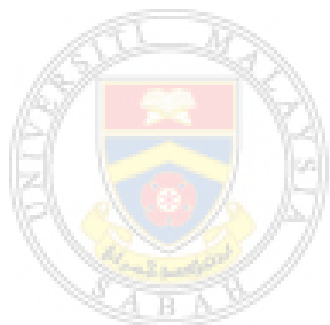
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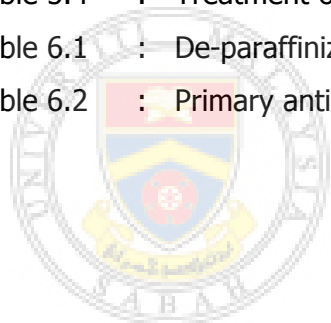
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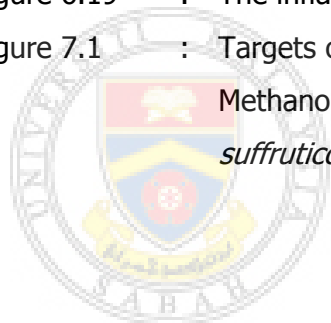
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ABBREVIATIONS

CCl₄	Carbon Tetrachloride
UV/ VIS	Ultraviolet/visible
GSH	Reduced glutathione
CAT	Catalase
Eq	Equation
NADP⁺	Oxidized nicotinamide adenine dinucleotide phosphate.
EDTA	Ethylene diamine tetra acetic acid.
LD₅₀	Lethal dose which cause the death of 50% of experimental animals
MDA	Malondialdehyde
b.w.	Body weight
Kg	Kilogram
L	Liter
Nmol	Nano mole
G	Gram
Ph	Potential of Hydrogen
µmole	Micro mole
ml	Milli liter
Mg	Milli gram
°C	Degree Celsius
M	Molar
Cm	Centimeter
USA	United State of America
NADPH	β-nicotinamide adenine dinucleotide 2 phosphate reduced tetrasodium salt
Min	Minimum
mM	Milli molar
QR	Quinone reductase
Vol	Volume
ROS	Reactive oxygen species
GR	Glutathion reductase
TNF	Tumor necrosis factor