

ABSTRACT

Tacca integrifolia Ker-Gawl is belongs to the family of Taccaceae and locally known as “Belimbing Tanah”. It has been used traditionally for the treatment of hypertension, hemorrhoids, heart failure and kidney disease. The thin layer chromatography (TLC) of hexane, petroleum ether, chloroform, methanol and water extract of leaves and rhizome showed the presence of phenols, flavonoids, terpenoids, essential oil and alkaloid compounds. The analysis of the leaves and rhizome extract with LCMS/MS showed that it contained proanthocyanidin, proanthocyanidin trimer, *p*-hydroxybenzoic acid, phenolic acid conjugate, protocatechuic acid, quinic acid and dicaffeolquinic acid conjugate. The total phenol contents were highest in leaves and rhizomes extract of water at 792.7mgGAE/g and 350.8mgGAE/g respectively. Whereas, the highest total flavonoids contents were highest in petroleum ether leaves extract and in chloroform rhizome extract at 376.7mgQE/g and 193.4mgQE/g respectively. The ACE inhibition activity at 100mg/ml was highest in leaves extract of water (45.5%) and in rhizome extract of methanol (53.6%). The isolated compounds label as A5, A7, A8, A9, B3, B5, B8, C3, C9, E1, E3, E4, F1, G1, G3 and G6 showed ACE inhibition more than 50%. Since the water extract showed highest ACE inhibition and because it is often used in traditional medicine for hypertension treatment, the water extracts were analyzed *in vivo* with SHR rats. The sub-acute toxicity test has shown that there were no mortality occurs under experimental conditions. Brine Shrimp Lethality Assay (BSLA) also has shown low toxicity at LC₅₀ 22981µg/ml for leaves extract and LC₅₀ 4378µg/ml of rhizome extract. The blood pressure of spontaneously hypertensive rats were reduced significantly ($p<0.05$) at 50mg/kg and 100mg/kg of water leaves extract and water rhizome extract respectively. The liver function test has indicated significant

difference in AST except when compared 100 mg/kg water rhizome extract SHR group with control group, and significance also showed in total protein and ALT except when compared 50 mg/kg water leaves extract and 50 mg/kg Captopril compared to control normal SD rat, respectively. While sodium test in renal function test showed significance in difference. In the antioxidant DPPH assay the IC_{50} of methanol leaves, chloroform leaves and water leaves extract was 88 μ g/ml, 350 μ g/ml and 480 μ g/ml respectively. The ferric reducing power assay has showed that both hexane extract from leaves and rhizome gave high reducing activity. The metal chelating activity of the chloroform leaves extract showed the highest metal chelating activity of IC_{50} at 1.98mg/ml. In conclusion, water extract from leaves and rhizome of *Tacca integrifolia* contained active phytochemical compounds as detected in LCMS/MS that responsible in reducing blood pressure of spontaneously hypertensive rats significantly at 50mg/kg and 100mg/kg and possessed high antioxidant activity that could scavenged free radicals and prevented oxidative stress that related to hypertension. Thus, these results support and provide scientific evidence to the claimed made by traditional practitioner that used *Tacca integrifolia* for antihypertension treatment.

ABSTRAK

Tacca integrifolia Ker-Gawl daripada keluarga Taccaceae juga dikenali sebagai "Belimbing Tanah". Ia telah digunakan secara tradisional untuk rawatan hipertensi, buasir, kegagalan jantung dan penyakit buah pinggang. Kromatografi lapisan nipis (TLC) pada ekstrak heksana, petroleum eter, klorofom, metanol dan air daripada daun dan rizom menunjukkan kehadiran sebatian fenol, flavonoid, terpenoid, minyak pati dan alkaloid. Analisis LCMS/MS ekstrak daun dan rizom menunjukkan bahawa ia mengandungi proantosianidin, trimer proantosianidin, asid *p*-hydroxybenzoic, konjugat asid fenolik, asid protocatechuic, asid quinic dan konjugat asid dicaffeolquinic. Jumlah kandungan fenol paling tertinggi dikesan dalam ekstrak air pada daun dan rizom iaitu sebanyak 792.7mgGAE/g dan 350.8mgGAE/g masing-masing. Manakala, jumlah kandungan flavonoid tertinggi dikesan di dalam ekstrak petroleum eter daun dan ekstrak kloroform rizom sebanyak 376.68mgQE/g dan 193.4mgQE/g masing-masing. Aktiviti perencatan ACE pada kepekatan 100mg/ml adalah tertinggi dalam ekstrak daun air (45.5%) dan dalam ekstrak rizom metanol (53.6%). Sebatian terpenoid berlabel A5, A7, A8, A9, B3, B5, B8, C3, C9, E1, E3, E4, F1, G1, G3 dan G6 menunjukkan perencatan ACE melebihi 50%. Oleh kerana ekstrak air menunjukkan perencatan ACE yang tertinggi dan sering digunakan oleh pengamal tradisional untuk rawatan hipertensi, maka ekstrak air dianalisis secara *in vivo* dengan menggunakan tikus SHR. Ujian ketoksikan sub-akut telah menunjukkan bahawa kematian tidak berlaku sepanjang eksperimen. Asei kematian anak udang (BSLA) juga telah menunjukkan ketoksikan adalah rendah pada LC₅₀ 22981.46µg/ml bagi ekstrak daun dan LC₅₀ 4378.011µg/ml bagi ekstrak rizom. Tekanan darah tikus hipertensi yang diberi dos ekstrak air daun dan rizom sebanyak 50mg/kg dan 100mg/kg telah berkurang dengan ketara ($p < 0.05$). Ujian fungsi hati telah menunjukkan tiada perbezaan yang signifikan dalam jumlah protein,

ALT dan AST. Begitu juga, dalam ujian fungsi renal, tiada perbezaan yang signifikan telah diperhatikan dalam kandungan natrium, kalium dan kreatinin apabila dibandingkan antara kumpulan tikus SHR kawalan dengan kumpulan tikus SHR yang diberi rawatan dengan ekstrak air daun dan ekstrak air rizom. Dalam asei antioksidan DPPH, IC_{50} ekstrak daun metanol, kloroform dan air adalah 88 μ g/ml, 350 μ g/ml dan 480 μ g/ml masing-masing. Asei pengurangan kuasa ferric telah menunjukkan bahawa kedua-dua ekstrak heksana daripada daun dan rizom memberikan aktiviti perencatan yang tinggi. Aktiviti pengkelat logam bagi ekstrak kloroform daun menunjukkan aktiviti perencatan tertinggi pada IC_{50} di 1.98mg/ml. Kesimpulannya, ekstrak air dari daun dan rizom *Tacca integrifolia* mengandungi sebatian fitokimia aktif yang dikesan dalam analisis LCMS/MS yang bertanggungjawab dalam mengurangkan tekanan darah tikus hipertensi secara ketara pada dos 50mg/kg dan 100mg/kg dan memiliki aktiviti antioksidan yang tinggi yang boleh menyingkirkan radikal bebas dan menghalang tekanan oksidatif yang berkaitan dengan tekanan darah tinggi. Oleh itu, hasil kajian ini menyokong dan menyediakan bukti saintifik seperti yang didakwa oleh pengamal tradisional yang menggunakan *Tacca integrifolia* sebagai rawatan hipertensi.

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LIST OF SYMBOLS AND ABBREVIATIONS

α -TOH	Alpha-Tocopherol
β -CAR	Beta-carotene
ACE	Angiotensin Converting Enzyme
ACEI	Angiotensin Converting Enzyme Inhibitor
AlCl ₃	Aluminum chloride
AP-I	activated protein-I
AscO ⁻	Ascorbate
AT1	Angiotensin II Type 1
AT2	Angiotensin II Type 2
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
BiONO ₃	Bismuth nitrate
Bp	Blood pressure
BSLA	Brine Shrimp Lethality Assay
CAT	catalase
CAP	Captopril
CVD	Cardiovascular Disease
DPPH	1,1-diphenyl-2-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
ENA	Enalapril
FeCl ₃	Ferric Chloride
GPX	glutathione peroxidase
H ₂ O ₂	Hydrogen peroxide
HA	Hippuric acid

HAc	Glacial acetic acid
HCl	Hydrochloric acid
HHL	Hippuryl-L-Histidyl-L-Leucine
HL	histidyl-L-leucine
HPLC	High Performance Liquid Chromatography
IC ₅₀	Half maximal Inhibitory concentration
K ⁺	Potassium
KI	Potassium Iodide
LC ₅₀	Median Lethal concentration
LCMS/MS	Liquid Chromatography Mass Spectrometry /Mass Spectrometry
LDL	Low Density Lipoprotein
MAPKs	mitogen-activated protein kinase
Na ⁺	Sodium
NaCl	sodium chloride
NADPH	Nicotinamide adenine dinucleotide Phosphate-oxidase
NANO ₃	Sodium nitrate
NaOH	Sodium hydroxide
NFκB	nuclear transcription factor kappa-β
NSAIDs	nonsteroidal anti-inflammatory drugs
O ₂	Oxygen
¹ O ₂	Single oxygen
O ₂ ^{••}	superoxide anion radicals
OD	Optical Density
OH [•]	Hydroxyl radicals
OS	Oxidative Stress

RAAS	Renin Angiotensin Aldosterone System
Rf	Retention factor
ROS	Reactive Oxygen Species
SHR	Spontaneously Hypertensive Rats
SOD	Superoxide dismutase
TCA	Trichloroacetic acid
TFA	Trifluoroacetic acid
TLC	Thin Layer Chromatography