Chemical and biological studies of the inflorescence of *Cocos nucifera* L., used in Ayurveda for the treatment of menorrhagia

by

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M. Phil. **2019**

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Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Master of Philosophy

Declaration of the Candidate

The work described in this thesis was carried out by me under the supervision of Dr. C. Padumadasa (principal supervisor), Prof. A. M. Abeysekera, Dr. B. Seneviratne, Dr. S. Padumadasa and Dr. M. G. Thammitiyagodage and report on this has not been submitted in whole or in part to any university or any other institution for anther Degree / Diploma.

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Declaration by the Supervisor

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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We certify that the candidate has incorporated corrections, additions and amendments recommended by the examiners in this version of the M. Phil. thesis.

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Abbreviations

Abbreviation	Explanation
AE	Aqueous extract
ABTS	2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
ALP	Alkaline phosphatase
AOAC	Association of official analytical chemist
ALT	Alanine transaminase
AQSPA	Aqueous soluble proanthocyanidins
AST	Aspartate aminotransferase
CHL/IU cells	Mammalian cell cultures
COX	Cyclooxygenase
CPCSEA	Committee for the purpose of control and supervision of experiments on animals
CTA	Chloramine-trichloroacetic acid
EASPA	Ethyl acetate soluble proanthocyanidins
EDTA	Ethylene diamine tetra acetate
DP	Degree of polymerization
DMAC	4-dimethylamino-cinnamaldehyde
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing ability of plasma
FLU-A	Influenza A
GC-MS	Gas chromatography couple to a mass spectrophotometer
GSPE	Seed extract of *Vitis vinifera*
HB	Haemoglobin

HPLC	High performance liquid chromatography
HCN	Prussic acid
HMB	Heavy menstrual bleeding
IDA	Iron deficiency anemia
LD_{50}	Median lethal dose
LOX	Lipoxygenase
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
NMR	Nuclear magnetic resonance
NP	Non-proanthocyanidin phenolic fraction
NP-PEG	Natural product-polyethelene glycol
IUPAC	International union of pure and applied chemistry
LNG-IUS	Levonorgestrel releasing intrauterine system
PIV	Parainfluenza virus
RBC	Red blood count
RDW-CV	Red blood cell distribution width
RSV	Respiratory syncytial virus
SV-40	simian virus 40
SAM	Shoot apical meristem
SPSS	Statistical package for social sciences
THP-1	Human monocytic cell line
TLC	Thin layer chromatography
MCP-1	Monocyte chemo attractant protein 1
MPV	Mean platelet volume
VCAM-1	Vascular cell adhesion protein 1

OECD	Organization for economic co-operation and development
PCT	Procalcitonin
ROW	Relative organ weight
RS	Ring system
WBC	White blood count

Acknowledgement

This research project was financially supported by the Research Grant; ASP/01/RE/SCI/25, University of Sri Jayewardenepura, Sri Lanka and without it, this work would not have been a success.

I would like to express my heartiest gratitude to my principal supervisor, Dr. C. Padumadasa who consistently allowed this project to be my own work, but guided me in the correct path whenever she thought I needed it. As well I would like to thank my supervisor, Prof (Emeritus). A. M. Abeysekera, for his precious and patient guidance to me for this research project. I would like to thank, my supervisors, Dr. M. G. Thammitiyagodage, Dr. S. Padumadasa and Dr. B. Seneviratne for their continuous advices to carry out this project.

My sincere thank also goes to technical officers in Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura who always assisted to carry out my laboratory work.

I express my profound gratitude to my colleagues, Dr. Ureshani Karunarathne, Dr. Kalpani Rathnayake, Thiloka Kariyawasam, Udari Kodithuwakku and Dr. Dayangi Hemalika who were always there for me with helping hands. As well, I am grateful to my parents, sister and brother for their unfailing support and continuous encouragement throughout my years of study. Finally, I would like to thank my loving husband, Eng. Subhash Dananjaya, without whose infinite patience, continuous encouragement and constant support, this could never be completed.

Thank you.

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ABSTRACT

Ayurvedic and traditional medical practitioners of Sri Lanka use the decoction of the immature inflorescence of *Cocos nucifera* L. variety aurantiaca for the treatment of menorrhagia. The extraction, purification, characterization and progestogenic effect of ethyl acetate soluble proanthocyanidins (EASPA) of this inflorescence have previously been reported. This finding is very significant as progestogens are widely used in the treatment of menorrhagia in western medicine.

EASPA, being a potential drug candidate in relation to treatment of menorrhagia was evaluated for its safety using acute and subacute toxicity studies in Wistar rats. Acute and subacute toxicity studies of EASPA were carried out according to OECD guidelines 423 and 407 respectively is reported herein. In the acute toxicity study, a single dose of EASPA (2000 mg/kg body weight) was orally administered to rats and monitored for 14 days. In the subacute toxicity study, rats were orally administered with EASPA daily for 28 days at doses of 1.75, 3.5, 7 and 14 mg/kg body weight. Rats in the acute or subacute toxicity study did not exhibit any mortality, clinical signs of toxicity, changes in haematological, biochemical and their histopathological investigations compared to those of control group rats. According to the results of acute toxicity, the LD_{50} of EASPA was estimated to be greater than 2000 mg/kg body weight. Considering the results of subacute toxicity study, it is possible to suggest that the oral administration of EASPA daily for 28 days was well tolerated up to the dose, 14 mg/kg in rats.

Acute and subacute toxicity of aqueous extract used in Ayurveda (AE) of the immature inflorescence of *Cocos nucifera* L. for the treatment of menorrhagia was evaluated for its safety in Wistar rats in order to compare the toxicity results with those of the EASPA. Acute and subacute toxicity studies of AE was carried out similar to those of EASPA. Dose levels of 150, 300, 600 and 1200 mg/kg body weight of AE were used for the subacute toxicity study of AE. All treated rats in both acute and subacute toxicity studies did not show any mortality or signs of toxicity. However, in the acute toxicity study, histopathological examination of liver of treated rats showed some signs of toxicity indicating hepatotoxic nature of this decoction at the tested dose. The LD_{50} of this AE was estimated to be greater than 2000 mg/kg body weight. Results of subacute toxicity study, suggest that the oral administration of AE daily for 28 days was well tolerated up to the dose, 1200 mg/kg in rats.

Cardiac glycosides are known as a major cardio toxins in plants. Therefore, investigation of the immature inflorescence of *Cocos nucifera* L. for the presence or absence of cardiac glycosides was carried out using chemical and chromatographic methods. According to results, cardiac glycosides were absent in this inflorescence. Proanthocyanidin content of the AE was determined using acid butanol method as an attempt to standardize it. The determined proanthocyanidin content of this AE was 619.6 mg in EASPA equivalence. Further, preliminary attempt to isolate phenolic compounds of the immature inflorescence of *Cocos nucifera* L. is reported herewith.