

**Reduction of cross contamination and  
extension of shelf life of ready to cook  
chicken meat treated with extracts of  
selected medicinal plants**

**By**

**Thikshani Somarathna**

**Ph.D.**

**2018**

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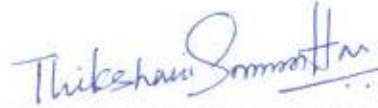


**Thesis submitted to the University of Sri Jayewardenepura  
for the award of the Degree of Doctor of Philosophy**

**2018**

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The work described in this thesis was carried out by me under the supervision of Dr. NS Weerakkody, Prof. KKDS Ranaweera, Dr. GAS Premakumara Dr. Mayuri Thammityagodage and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree "Diploma".



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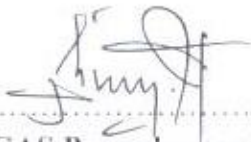
.....  
**Dr. NS Weerakkody**  
Principle Supervisor  
Senior Lecturer,  
Department of Agricultural and  
Plantation Engineering,  
Faculty of Engineering Technology,  
The Open University of Sri Lanka

Date .. 17/05/2019 .....



.....  
**Prof. KKBS Ranaweera**  
Internal- Supervisor  
Senior Professor,  
Department of Food Science and Technology,  
Faculty of Applied Science,  
University of Sri Jayewardenepura,  
Sri Lanka

Date .. 17/05/2019 .....



.....  
**Dr. GAS Premakumara**  
External- Supervisor  
Director,  
Industrial Technology Institute,  
363, Buddaloka Mawatha  
Colombo 7, Sri Lanka

Date .. 17/05/2019 .....



.....  
**Dr. Mayuri Thanmitiyagodage**  
External-Supervisor  
Head/ Animal Center,  
Medical Research Institute,  
Borella, Colombo, Sri Lanka

Date .. 17/05/2019 .....

**M. G. Thammitiyagodage** B.V.Sc. M.Phil.  
Veterinary Surgeon  
Medical Research Institution  
Colombo.

## Certification of the Supervisors

We certify that the the candidate has incorporated all corrections, addition and ammenmendts recommended by the examiners to this version of the PhD thesis.



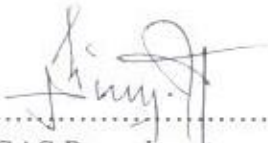
Dr. NS Weerakkody  
Principle Supervisor  
Senior Lecturer,  
Department of Agricultural and  
Plantation Engineering,  
Faculty of Engineering Technology,  
The Open University of Sri Lanka

Date ..17/05/2019.....



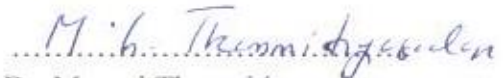
Prof. KKDS Ranaweera  
Internal- Supervisor  
Senior Professor,  
Department of Food Science and Technology,  
Faculty of Applied Science,  
University of Sri Jayewardenepura,  
Sri Lanka

Date ..17/05/2019.....



Dr. GAS Premakumara  
External- Supervisor  
Director/Senior Research Fellow,  
Industrial Technology Institute,  
363, Buddaloka Mawatha  
Colombo 7, Sri Lanka

Date ..17/05/2019.....



Dr. Mayuri Thanmitiyagodage  
External-Supervisor  
Head/ Animal Center,  
Medical Research Institute,  
Borella, Colombo, Sri Lanka

Date ..17/05/2019.....

M. G. Thanmitiyagodage B.V.Sc. M.Phil.  
Veterinary Microbiologist  
Medical Research Institution  
Colombo.

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## Abbreviations

ABTS	2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)
A549	Lung carcinoma cell line
ACA	Acetoxychavicol acetate
AC	Acridine orange
ANOVA	Analysis of variance
ATCC	American type culture collection
BHI	Brain heart infusion
BHA	Butylated hydroxyanisol
BHT	Butylated hydroxytoluene
BPA	Baird-Parker agar
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
COS7	Monkey Fibroblast Cell Line
COSY	Correlation Spectroscopy
CORL-23	Lung Carcinoma Cell Line
CLSL	Clinical Laboratory Standard Institute
DEPT	Distortionless enhancement by polarization transfer
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's Modified Eagle's medium
DIZ	Diameter of inhibition zone
EO	Essential oils
EB	Ethidium Bromide
EDTA	Ethylenediaminetetraacetic acid
ETEC	Enterotoxigenic <i>Escherichia coli</i>
ET	Electron transfer
ESE	Enhance Solvent Extraction
FAO	Food and Agricultural Organisation
FBS	Fetal Bovine Serum
FCR	Folin-Ciocalteus reagent
GAE	Gallic acid equivalent
GC-MS	Gas chromatography-mass spectrometry

HAT	Hydrogen Trasfer
HCA	Hydroxycitric acid
HHP	High hydrostatic pressure
HMBC	Heteronuclear Multiple Bond Correlation
HePG2	Hepatocellular carcinoma cell line
HSPE	High Pressure Solvent Extraction
MDA	Malondialdehyde
MAP	Modified atmosphere packaging
MDR	Multiple drug resistance
MH	Mueller Hinton
MIC	Minimum inhibitory concentration
MLC	Minimum lethal concentration
MPN	Most probable number
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin sensitive <i>Staphylococcus aureus</i>
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCCS	National Center for Cell Science
NMR	Nuclear magnetic resonance spectroscopy
NOEAL	No observed Adveced Effect Level
OD	Optical density
OECD	Organization for Economic Cooperation and Development
ORAC	Oxygen Radical Absorbance Capacity
PALCAM	Polymyxin Acriflavine Lithium Chloride Ceftazidime
PBS	Phosphate Buffered Solution
PC3	Prostate adenocarcinoma cell line
PCA	Plate count agar
PLE	Pressured Liquied Extraction
PV	Peroxide value
ROS	Reactive Oxygen Species
RBC	Red Blood Count
RTE	Ready to Cook
SEM	Scanining electron microscopy

TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TPC	Total phenolic content
TLC	Thin layer chromatography
TSA	Tryptic soy agar
TSB	Tryptic soy broth
VP	Vacuum pack
XOD	Xanthine oxidase
XLD	Desoxycholate agar
VRBG	Violet red bile glucose agar
WBC	White blood count



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## **Reduction of cross contamination and extension of shelf life of ready to cook chicken meat treated with extracts of selected medicinal plants**

**Thikshani Somarathna**

### **ABSTRACT**

The aim of this study was to investigate the antimicrobial activity of less-utilized plants against food-borne pathogens *Escherichia coli*, *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Staphylococcus aureus*, and to identify possible toxicity limits for safe use for extending the shelf life of ready to cook chicken meat.

In this study, four underutilized plants including *Alpinia malaccensis* (“Ran- kihiria”), *Terminalia catappa* (“Kottamba”), *Alpinia purpurata* (“Niyapothu mal”) and *Curcuarma zedoaria* (“Haran-kaha”) were extracted using ethanol or hexane solvents.

The hexane rhizome extract of *A. malaccensis* showed significantly ( $P < 0.05$ ) higher diameter of inhibition zone ranging from  $33.0 \pm 1.41$  to  $40.3 \pm 0.42$  mm against *S. aureus* compared to other extracts. The lowest Minimal Inhibition Concentration (MIC) and Minimal Bactericidal Concentration (MBC) against *S. aureus* which was 20 and 80 mg/ml, respectively. GC-MS analysis showed an 82.87 % a major chemical compound for *A. malaccensis* whereas for *T. catappa* it was 2, 5-Furandione, 3- methyl (32.23%). The purified active fraction of *A. malaccensis* was identified by TLC bio-autography and it was confirmed as 1’Acetoxychavicol Acetate (1’ ACA) by Nuclear Magnetic Resonance.

The combined plant extract of *A. malaccensis* (2.5 mg/ml) and *T. catappa* (20 mg/ml) had significant ( $P < 0.05$ ) synergistic antibacterial activity against *S. aureus* and *L. monocytogenes*. Also observed significant antibiofilm activity of *A. malaccensis* for both of cultures. The most effective combination was 5 mg/ml of *A. malaccensis* with 20 mg/ml of *T. catappa* for the antibiofilm activity.

*A. malaccensis* and *T. catappa* were used to evaluate the acute oral toxicity using Wistar rats and cytotoxicity study using cell culture techniques AO/EB, Hoechst staining and comet assay for A549, HepG2, 3T3 and COS7 cell lines. The acute oral toxicity studies for both extracts at a single dose of 2000 mg/kg body weight did not cause any lethality or produce any remarkable changes in general behavior, body weights, biochemical parameters and histopathological studies. In addition, cytotoxicity results showed that non-toxic concentrations of *A. malaccensis* (2, 1.4, 30 and 1.4 µg/ml) and *T. catappa* (300, 300 and 130 µg/ml) were not mediated apoptotic cell death or necrosis, or DNA damage. Calculated approximately Annual Daily Intake for *A. malaccensis* and *T. catappa* were 55.41g/day and 1549.70 mg/day. Therefore, the therapeutic levels for antibacterial activity of the plant extracts could be safe for consumption.

Finally, the use of both plant extracts in controlling *L. monocytogenes*, *S. aureus* and spoilage bacteria in vacuum packed ready-to-cook (RTC) marinated chicken was evaluated. Combination of both plant extracts significantly ( $p < 0.05$ ) inhibited the growth of *S. aureus* with 1.80, 2.13, 2.36 and 2.97 log cfu/g reduction over 3, 6, 9 and 12 days at 8 °C. Similarly, *L. monocytogenes* was significantly ( $p < 0.05$ ) inhibited at 6, 9, 12 days with 1.22, 1.60 and 1.55 log cfu/g reduction compared to control at 4 °C. Both temperatures significantly reduced ( $P < 0.05$ ) lipid oxidation in treated chicken compared to control. The shelf life of RTC marinated vacuum packed fresh chicken samples stored at 4 and 8 °C were significantly extended for 6 and 9 days respectively. Therefore, both plants extracts combination could use as antimicrobials and antioxidants in extending shelf life of RTC vacuum packed fresh chicken meat products.

**Key words:** Underutilized plant, Foodborne pathogens, *A. malaccensis*, 1'ACA, Chicken