

**STUDIES ON LOCAL MANGO- AND NONI-
DERIVED MILK CURDS AND THEIR
BIOLOGICAL ACTIVITIES**

NORLIANA BINTI MUNIR

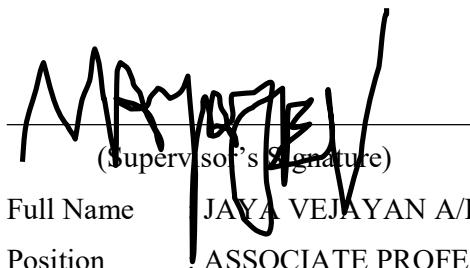
MASTER OF SCIENCE

UNIVERSITI MALAYSIA PAHANG



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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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NORLIANA BINTI MUNIR

Thesis submitted in fulfillment of the requirements
for the award of the degree of
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ABSTRACT

The dairy market has grown favourably with constant growth rates in Malaysia in the past years. However, certain controversies surrounding the use of calf rennet as milk coagulant has encouraged the search for its alternatives, especially of plant origins. Hence, the study aims to investigate local plants having a dual effect of coagulating and fortifying milk simultaneously. In this study, ten samples from nine plants have been screened for the best two potential rapid milk coagulant. A total of three plants exhibited positive results on milk coagulation, with *M. citrifolia* juice extract showed the fastest coagulation time (10.8 ± 0.36 s) followed by *M. indica* seed kernel extract (28.8 ± 0.61 s) and *T. catappa* leaf extract (40.73 ± 1.91 s). Two plants proceeded with the further tests of determining the coagulant action by investigating the protein presence within extract through size exclusion chromatography, Bradford protein assay, SDS-PAGE analysis, and LCMS analysis. Both plants were proven to coagulate milk by enzyme action, with *M. citrifolia* was having definite proof of possessing protease-like enzyme (cysteine protease) with a molecular size of approximately 30 kDa. Then, the milk curd derived from both extracts of *M. indica* and *M. citrifolia* were further tested on their antioxidant and antidiabetic properties. The assays of TPC, DPPH, and FRAP were performed in determining the antioxidant activity of the milk curd. The result showed *M. indica*-added milk curd was having higher total phenolic content (34.7 ± 0.49 mg GAE/g) and antioxidant capacity of FRAP assay (317.16 ± 0.99 mg GAE/g), and the lower IC₅₀ value for DPPH assay (12.31 ± 0.66 µg/mL) compared to *M. citrifolia*-added milk curd. Antidiabetic properties were assessed via α-amylase and α-glucosidase *in vitro* inhibitory activity assays. *M. indica*-added milk curd shown to have lower IC₅₀ value in both assays compared to *M. citrifolia*-added milk curd, with 354.6 ± 20.26 µg/mL for α-amylase inhibitory assay and 4.86 ± 0.16 mg/mL for α-glucosidase inhibitory assay. Based on overall evaluations, *M. indica* was a better coagulant and fortifier of biological activities than *M. citrifolia*, and both plants found with the added advantage of being a fortifier compared to the rennet of only able to coagulate milk. The study showed the potential of local plants as milk coagulants as well as having benefits of biological activities and if developed into yogurt or cheese products, able to encourage the local dairy industry.

ABSTRAK

Pasaran tenusu telah berkembang dengan baik dengan kadar pertumbuhan yang berterusan di Malaysia pada tahun-tahun kebelakangan ini. Namun, kontroversi tertentu mengenai penggunaan rennet daripada anak lembu sebagai koagulan susu telah mendorong kepada pencarian alternatif, terutama yang berasal dari tumbuhan. Oleh itu, kajian ini bertujuan untuk mengkaji tumbuhan lokal yang mempunyai kesan berganda, iaitu penggumpalan dan menguatkan susu secara serentak. Dalam kajian ini, sepuluh sampel dari sembilan tumbuhan telah dikaji untuk mendapatkan dua penggumpal susu pantas yang berpotensi. Sebanyak tiga tumbuhan menunjukkan hasil positif pada penggumpalan susu, dengan ekstrak jus *M. citrifolia* menunjukkan masa penggumpalan terpantas (10.8 ± 0.36 s) diikuti dengan ekstrak biji *M. indica* (28.8 ± 0.61 s) dan ekstrak daun *T. catappa* (40.73 ± 1.91 s). Dua tumbuhan diteruskan ujian selanjutnya untuk menentukan tindakan penggumpalan dengan menyiasat kehadiran protein dalam ekstrak melalui kromatografi pengecualian saiz, analisis protein Bradford, analisis SDS-PAGE, dan analisis LCMS. Kedua tanaman terbukti menggumpal susu melalui tindakan enzim, dengan *M. citrifolia* memiliki bukti kukuh memiliki enzim mirip protease (cysteine protease) dengan ukuran molekul sekitar 30 kDa. Kemudian, dadih susu yang berasal dari kedua ekstrak *M. indica* dan *M. citrifolia* diuji lebih lanjut mengenai sifat antioksidan dan antidiabetiknya. Pengujian TPC, DPPH, dan FRAP dilakukan dalam menentukan aktiviti antioksidan dadih susu. Hasilnya menunjukkan dadih susu ditambah *M. indica* mempunyai kandungan fenolik total yang lebih tinggi (34.7 ± 0.49 mg GAE/g) dan kapasiti antioksidan ujian FRAP (317.16 ± 0.99 mg GAE/g), dan nilai IC₅₀ yang lebih rendah untuk ujian DPPH (12.31 ± 0.66 µg/mL) berbanding dadih susu yang ditambah *M. citrifolia*. Sifat antidiabetik dinilai melalui ujian aktiviti perencatan α-amilase dan α-glukosidase secara *in vitro*. Dadih susu ditambah *M. indica* menunjukkan nilai IC₅₀ yang lebih rendah dalam kedua-dua ujian berbanding dadih susu ditambah *M. citrifolia*, dengan 354.6 ± 20.26 µg/mL untuk ujian perencatan α-amilase dan 4.86 ± 0.16 mg/mL untuk ujian perencatan α-glukosidase. Berdasarkan penilaian keseluruhan, *M. indica* adalah penggumpal dan penguat aktiviti biologi yang lebih baik berbanding *M. citrifolia*, dan kedua-dua tumbuhan didapati mempunyai kelebihan tambahan sebagai penguat berbanding dengan rennet yang hanya mampu menggumpal susu. Kajian menunjukkan potensi tumbuhan tempatan sebagai koagulan susu serta mempunyai manfaat aktiviti biologi dan jika dikembangkan menjadi produk yogurt atau keju, dapat mendorong industri tenusu tempatan.

TABLE OF CONTENT

SUPERVISOR'S DECLARATION

STUDENT'S DECLARATION

TITLE PAGE

ACKNOWLEDGEMENTS	ii
-------------------------	----

ABSTRACT	iii
-----------------	-----

ABSTRAK	iv
----------------	----

TABLE OF CONTENTS	v
--------------------------	---

LIST OF TABLES	viii
-----------------------	------

LIST OF FIGURES	ix
------------------------	----

LIST OF SYMBOLS	xi
------------------------	----

LIST OF ABBREVIATION	xiii
-----------------------------	------

LIST OF APPENDICES	xv
---------------------------	----

CHAPTER 1: INTRODUCTION

1.1 Research Background	1
1.2 Problem Statement	3
1.3 Objectives	4
1.4 Scope of Research	4
1.5 Significance of Research	5

CHAPTER 2: LITERATURE REVIEW

2.1 Background of Selected Plant Based in their Biological Activities	6
2.2 Coagulation of Milk	11
2.2.1 Enzyme-mediated Coagulation	12
2.2.2 Acid-mediated Coagulation	13

2.2.3	Factors Affecting Milk Coagulation	14
2.2.4	Coagulant – Rennet and Plant	16
2.2.5	Milk Coagulated Products – Cheese and Yogurt	23
2.3	Techniques and Applications	24
2.3.1	Size Exclusion Liquid Chromatography for Fractionation of Protein	24
2.3.2	Bradford Protein Assay for the Determination of Protein Concentration	28
2.3.3	SDS-PAGE for Protein Identification	30
2.3.4	Reaction Assays for the Determination of Antioxidant Activity	31
2.3.5	Reaction assays for the Determination of Antidiabetic Activity	35

CHAPTER 3: MATERIALS AND METHODOLOGY

3.1	Introduction	37
3.2	Materials	39
3.3	Collection, Preparation, Extraction, and Screening of Plant Extracts	40
3.3.1	Sample Collection	40
3.3.2	Sample Preparation	41
3.3.3	Extraction of Plant Materials	42
3.3.4	Screening of Milk Coagulation Capability in Selected Plant Materials	43
3.4	Classification of the Plant Coagulant of <i>M. indica</i> and <i>M. citrifolia</i>	44
3.4.1	Determination of Protein Presence within Plant Extracts	44
3.4.2	Investigations of Plant Coagulants	47
3.5	Determination of Biological Activities of Fortified Curds	52
3.5.1	Determination of Optimum Condition for Curd Preparation	52
3.5.2	Determination of Antioxidant Properties	54

3.5.3 Determination of Antidiabetic Properties	56
3.5.4 LCMS Analysis of Curd for Biologically Significant Micro-constituents	57

CHAPTER 4: RESULT AND DISCUSSION

4.1 Introduction	59
4.2 Sample Collection, Preparation, Extraction, and Screening of Plants	59
4.2.1 Sample Collection, Preparation, and Extraction	59
4.2.2 Screening of Milk Coagulation Capabilities in Selected Plant Materials	62
4.3 Classification of the Plant Coagulants of <i>M. indica</i> and <i>M. citrifolia</i>	66
4.3.1 Determination of Protein Presence within Plant Extracts	66
4.3.2 Investigations of Plant Coagulants	72
4.4 Determination of Biological Activities of Fortified Curds	81
4.4.1 Optimum Conditions for Curd Preparation	81
4.4.2 Determination of Antioxidant Properties	90
4.4.3 Determination of Antidiabetic Properties	101
4.4.4 LCMS Analysis of Curd for Biologically Significant Micro-constituents	105
4.5 Challenges of having Plant Enzymatic Coagulant versus Curd with Potent Biological Activities	106

CHAPTER 5: CONCLUSION

5.1 Conclusion	109
5.2 Recommendations	110

REFERENCES

APPENDICES

REFERENCES

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