



POTENTIAL NATURAL SURFACTANTS FROM PLANT
EXTRACTS FOR THE PREPARATION
OF PHARMACEUTICAL EMULSIONS AND THEIR
ANTIMICROBIAL PROPERTIES

BY

JANAN NIMA HADI

A thesis submitted in fulfilment of the requirement
for the degree of Master in Pharmaceutical Sciences
(Pharmaceutical Technology)

Kulliyyah of Pharmacy

International Islamic University
Malaysia

JULY 2010

ABSTRACT

Amphiphilic molecules play a key role in the stabilization of many of the colloids. It is, therefore, very important to understand the interfacial behaviour of these molecules and to select the proper ones for the proper activity. Synthetic surfactants and emulsifiers are widely used in many of our foods and pharmaceutical formulation, but, it becomes very important to replace them by natural molecules with good health records. Five medicinal plants which are *Syzygium aromaticum*, *Entada spiralis*, *Trigonella foenum-graecum*, *Elephantopus scaber* and *Andrographis paniculata* were selected for this study. The crude extract of the plants were prepared by maceration method. Solvents with different polarity were used for the extraction. The physical properties, in particular the surface activity of the extracts were evaluated and compared. Properties of emulsions prepared from the crude extracts were then investigated. Homogenization was carried out from 20% palm oil with 10% crude extract. The antimicrobial activities of the extracts against two Gram-positive *Bacillus subtilis* and *Staphylococcus aureus* and two Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* were investigated. Both the disc diffusion (qualitative) and tube macrodilution (quantitative) assays were employed for the determination of antimicrobial activity. The extracts of *E. spiralis* and *S. aromaticum* from ethanol-water 1:1 gave stable emulsions at least up to six months when kept at room temperature. The surface active compounds, if present among the components extracted will be adsorbed differently at the interface producing different extent of emulsion stability. All extracts were able to inhibit the growth of one or more of the bacteria. The patterns of inhibition varied with the type of plant extract, the solvent used for extraction and the organism tested. *S. aureus*, was the most susceptible to all plant extracts while *E. coli* was the most resistant microorganism. The highest antibacterial activity was observed from *S. aromaticum* extract with lowest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 0.39 mg/mL and 0.78 mg/mL, respectively against *B. subtilis*. It can be concluded that the extracts from *S. aromaticum* and *E. spiralis* have the potential to be used for the preparation of stable pharmaceutical emulsions by providing both emulsifying and antimicrobial actions.

ملخص البحث

ان الجزيئات ذات المجاميع الموجبة والسالبة تلعب دور اساسي في تثبيت عدد من المحاليل الغروية, ولهذا يكون من المهم معرفة سلوك هذه الجزيئات وانتقاء الاكثر فعالية منها عند تماس السطوح غير المتجانسة لاختيار جزيئات ذات النشاط الملائم. ان المواد الخافضة للشد السطحي (surfactants) والمستحلبات الصناعية قد استعملت بشكل كبير في الصناعات الغذائية والدوائية الا ان استبدال هذه المواد الصناعية بمواد طبيعية مهم من الناحية الصحية والغذائية. وقد تم استخدام خمس نباتات طبية في هذه الدراسة وهي (*Syzygium aromaticum*, *Entada spiralis* *Trigonella foenum-graecum*, *Elephantopus scaber* و *Andrographis paniculata*). ان المستخلصات الخام لهذه النباتات قد حضرت بطريقة التنقيح واستخدمت كذلك مذيبات ذات قطبية مختلفة للاستخلاص. ان الخواص الفيزيائية وخاصة خاصية الشد السطحي لهذه العصارات وتأثيرها على السوائل قد سجلت وقورنت مع خواص مستحلبات نباتات اخرى. وقد تم تحضير المستحلبات من الخلط المتجانس بنسبة 20% من زيت النخيل و 10% من المستخلص الخام للنباتات و 70% من الماء المقطر لمرتين. وتم في هذه الدراسة البحث عن تأثير المستخلصات النباتية على فعالية بعض انواع البكتريا المرضية الموجبة لصبغة كرام وهي (*Bacillus subtilis* و *Staphylococcus aureus*) والسالبة لصبغة كرام وهي (*Escherichia coli*, *Pseudomonas aeruginosa*) بطريقتين بحثية وهما (disc diffusion) و (tube macrodilution). ان المستحلب المحضر من المستخلصات الخام المحضرة من النباتات التالية (*S. aromaticum* و *E. spiralis*) المستخلصة من الماء – الايثانول بنسبة 1-1 قد اعطى مستحلب ثابت لمدة ستة اشهر في درجة حرارة الغرفة. ان تأثير العصارة الخام من النباتات على الشد السطحي للسوائل قد وجدت انها تمتاز بدرجات مختلفة بين سطحين غير متجانسين من السوائل (زيت وماء) معطية مستحلبات مستقرة بدرجات متفاوتة كما تم ملاحظة ان كل العصارات النباتية كان لها تأثير على النشاط البكتيري. كما ان هذه التأثيرات المختلفة تعتمد على نوع العصارة الخام والمذيب المستخدم وقد بين ان بكتيريا (*S. aureus*) كانت اكثر حساسة للعصارات النباتية بينما بكتيريا (*E. coli*) كانت الاكثر مقاومة. وكانت عصارة (*S. aromaticum*) الاعلى تأثيراً على نشاط البكتيريا المستخدمة في البحث. وبين عند حساب (MIC) و (MBC) لهذه العصارة النباتية ان التراكيز التالية (0.39 و 0.78 mg/mL) هي الاكثر تأثير على النشاط البكتيري لبكتيريا (*B. subtilis*) وبين من نتائج البحث ان من الممكن تحضير مستحلبات صيدلانية طبيعية مستقرة من عصارة النباتات الاتية (*S. aromaticum* و *E. spiralis*).

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion; it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

.....
Kausar binti Ahmad
Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

.....
Norazian binti Mohd Hassan
Co supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

.....
Maryanto
Examiner

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

.....
Sundany
Examiner

This thesis was submitted to the Department of Pharmaceutical Technology and was accepted as a fulfilment of the requirements for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

.....
Juliana binti Md Jaffri
Head, Department of Pharmaceutical
Technology

This thesis was submitted to the Kulliyyah of Pharmacy and was accepted as a fulfilment of the requirements for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

.....
Tariq bin Abdul Razak
Dean, Kulliyyah of Pharmacy

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Janan Nima Hadi

Signature.....

Date.....

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

**DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE
OF UNPUBLISHED RESEARCH**

Copyright ©2010 by Janan Nima Hadi. All rights reserved.

**POTENTIAL NATURAL SURFACTANTS FROM PLANT
EXTRACTS FOR THE PREPARATION
OF PHARMACEUTICAL EMULSIONS AND THEIR
ANTIMICROBIAL PROPERTIES**

No part of this unpublished research may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise without the prior written permission of the copyright holder except as provided below.

1. Any material contained in or derived from this unpublished research may only be used by others in their writing with due acknowledgement.
2. IIUM or its library will have the right to make and transmit copies (print or electronic) for institutional and academic purposes.
3. The IIUM library will have the right to make, store in a retrieval system and supply copies of this unpublished research if requested by other universities and research libraries.

Affirmed by Janan Nima Hadi.

.....

Signature

.....

Date

ACKNOWLEDGEMENTS

All praise is due to Allah S.W.T because of His bounty I can complete this research towards fulfilling the requirements for my master degree.

First I would like to express my most sincere gratitude to my supervisor, Assistant Professor Dr. Kausar Ahmad and for my co-supervisor, Assistant Professor Dr Azian for their help and guidance.

I offer my regards and blessings to all of those from Kulliyyah of Pharmacy and medicine, International Islamic University Malaysia, Kuantan who supported me in any respect during the completion of the project.

I would like to express my utmost gratitude to my family: my husband and my children for their moral support that gave me the confidence, will and strength to endure pressure and tension in pursuing my ambition to complete this work.

Last but not least to my parents, sisters, and brother for being supporting me all the time.

TABLE OF CONTENT

Abstract	ii
Abstract in Arabic	iii
Approval Page.....	iv
Declaration	vi
Declaration of Copyright	vii
Acknowledgements.....	viii
List of Tables	xii
List of Figures	xiii
List of Abbreviations	xvi

PART 1 : INTERFACIAL ACTIVITY AND EMULSION FORMATION USING CRUDE EXTRACTS OF MEDICINAL PLANTS AS EMULSIFIERS..... 1

CHAPTER 1: INTRODUCTION.....	2
1.1 Plants used in this study	2
1.1.1 Fenugreek (<i>Trigonella foenum -graecum</i>)	2
1.1.2 Clove (<i>Syzygium aromaticum</i>)	5
1.1.3 Hempedu Bumi (<i>Andrographis paniculata</i>)	7
1.1.4 Tapak Leman (<i>Elephantopus scaber</i>).....	8
1.1.5 Beluru (<i>Entada spiralis</i>)	9
1.2 Plant Extraction.....	10
1.3 Surfactans.....	11
1.4 Natural and Synthetic Surfactants.....	13
1.5 Surface Tension	17
1.6 Critical Micelle Concentration (CMC)	18
1.7 Interfacial Tension	20
1.8 Emulsion	21
1.8.1 Stable and unstable emulsion.....	24
1.8.2 Applications of emulsion	27
1.9 Aims of the Study	28

CHAPTER 2: MATERIALS AND METHODS	29
2.1 Materials and Chemicals.....	29
2.1.1 Plants.....	29
2.1.2 Chemicals.....	29
2.1.3 Palm Oil	29
2.2 Methods.....	30
2.2.1 Extraction of plants	30

2.2.1.1 Ethanol/double distilled water 1:1 (v/v) extract.....	30
2.2.1.2 Ethanol/double distilled water 9:1 (v/v) extract.....	31
2.2.1.3 Aqueous extract.	31
2.2.1.4 Chloroform/ Isopropanol 1:1 (v/v) extract.....	31
2.2.2 Surface tension and interfacial tension measurement	32
2.2.2.1 Determination of surface tension	32
2.2.2.2 Determination of critical micelle concentration (cmc)	33
2.2.2.3 Sample preparation for interfacial tension	33
2.2.3 Preparation of emulsions.....	34
2.2.3.1 Particle size determination	35
2.2.3.2 Phase separation.....	36
CHAPTER 3: RESULTS	37
3.1 Surface Tension	37
3.1.1 S.aromaticum (Clove)	38
3.1.2 E. spiralis (Beluru)	38
3.1.3 Foenum-graecum (Fenugreek).....	39
3.1.4 E. scaber (Tapak leman).....	39
3.1.5 A. paniculata (Hempudu Bumi).....	40
3.2 Characterization of Emulsion	45
3.2.1 S. aromaticum (Clove)	46
3.2.2 E. spiralis (Beluru)	46
3.2.3 T. foenum-graecum (Fenugreek)	46
3.2.4 E. scaber (Tapak leman)	47
3.2.5 A. paniculata (Hempudu bumi).....	47
CHAPTER 4: DISCUSSION	54
4.1 Crude Extract	54
4.2 Surface Properties	59
4.3 Emulsification Properties.....	62
4.4 Conclusion	62
PART 2: ANTIMICROBIAL PROPERTIES OF SELECTED MEDICINAL PLANT	64
CHAPTER 1	65
1.1 Introduction.....	65
1.2 Aim	69
CHAPTER 2: MATERIALS AND METHODS :	70
2.1 Plant Materials	70
2.2 Microorganisms	70
2.3 Preparation of Plant Extracts	70
2.4 Maintenance of Microorganisms	70

2.5 Inoculums Preparations.....	70
2.6 Antimicrobial Activity Screening Test	71
2.7 Quantitative Antimicrobial Activity Test	71
2.7.1 Determination of Minimum Inhibitory Concentration (MIC) ...	71
2.7.2 Determination of Minimum Bactericidal Concentration (MBC)	73
CHAPTER 3: RESULTS	74
CHAPTER 4: DISCUSSION AND CONCLUSION	83
4.1 Discussion	83
4.2 Conclusion	88
BIBLIOGRAPHY	89
APPENDIX A: PLANT PHOTOS USED IN THIS STUDY	103
APPENDIX B: THE EFFECTS OF VARIOUS CONCENTRATIONS OF CRUDE PLANTS EXTRACTS ON SURFACE TENSION, INTERFACIAL TENSION (PART ONE).	106
APPENDIX C: THE EFFECT OF USING CRUDE PLANT EXTRACT ON THE PREPARATION OF EMULSION	111
APPENDIX D: EMULSION USING NATURAL EMULSIFIER	120
APPENDIX E: REPORTED RESULTS FOR ANTIBACTERIAL ACTIVITIES OF CIPROFLOXACIN PART TWO	127
APPENDIX F: MAIN PHYTOCHEMICALS OF PLANT EXTRACTS STUDIED.	128
APPENDIX G: ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS STUDIED.	130
APPENDIX H: ABSTRACT APPEARED IN THE ABSTRACT BOOK OF THE 8TH MPS PHARMACY SCIENTIFIC CONFERENCE (15TH – 17TH AUGUST 2008), KUANTAN, MALAYSIA	133

LIST OF TABLES

<u>Table No</u>	Part 1	<u>Page No</u>
1.1	Chemical components of <i>T. foenum</i> seed.	3
2.1	Plant materials	29
2.2	Properties of palm oil	30
3.1	Surface activity of plant extract	41
3.2	Particle size of oil droplets after one hour.	53
	Part 2	
3.1	Antibacterial activities of various plant extracts screened by using disc diffusion assay.	76
3.2	Minimum Inhibitory Concentration (MIC, mg/ml) and Minimum Bactericidal Concentration (MBC, mg/ml) of the plant extracts against the tested microorganisms.	82

LIST OF FIGURES

<u>Figure No</u>		<u>Page No</u>
1.1	Structure of Galactomannan.	4
1.2	Structure of Eugenol	6
1.3	Structure of Andrographolide	7
1.4	Major chemical constituents of leaf oils of <i>E. Scaber</i> (a) hexadecanoic acid (b) octadecadienoic	8
1.5	Chemical structure of alpha-solanine, an example of a monodesmosidic, branched-chain steroidal saponin.	10
1.6	Production of petrochemicals and oleo chemicals.	14
1.7	Structure of different classes of surfactants.	15
1.8	The interactions of a molecule in the bulk of a liquid.	18
1.9	Surface active molecules absorbed at the air/water interface, decreasing surface tension	19
1.10	Effect of surfactant concentration on surface tension	20
2.1	Du Noüy tensiometer	32
2.2	Calculation of critical micellar concentration value from the surface tension vs. concentration plot.	33
2.3	Polyoxyethylene 20 sorbitan monoester (Tween [®])	34
2.4	Ultra-Turrax [®] T10 Basic homogeniser (Ika Labortechnik, Germany).	35
3.1	(a) Effect of concentration of ethanol-water 1:1 crude extract on the surface tension of water.	42
	(b) Effect of concentration of ethanol-water 9:1 crude extract on the surface tension of water.	42
	(c) Effect of concentration of chloroform-isopropanol crude extract on the surface tension of oil.	43
	(d) Effect of concentration of aqueous crude extract on the surface tension of water.	45

3.2	(a) Effect of ethanol-water 1:1 crude extract on the oil-water interfacial tension.	44
	(b) Effect of ethanol-water 9:1 crude extract on the oil-water interfacial tension.	44
	(c) Effect of chloroform-isopropanol 1:1 crude extract on the oil-water interfacial tension.	45
	(d) Effect of aqueous crude extract on the oil-water interfacial tension.	45
3.3	Phase separation of emulsion prepared using crude extract from <i>S. aromaticum</i> .	48
3.4	Particle size of oil droplets stabilized by extracts from <i>S. aromaticum</i> . (a) D (0.5) and (b) D (0.9).	48
3.5	Phase separation of emulsion prepared using crude extract from <i>E. Spiralis</i> .	49
3.6	Particle size of oil droplets stabilized by extracts from <i>E. Spiralis</i> . (a) D (0.5) and (b) D (0.9).	49
3.7	Phase separation of emulsion prepared using crude extract from <i>T. foenum</i> .	50
3.8	Particle size of oil droplets stabilized by extracts from <i>T. foenum</i> . (a) D (0.5) and (b) D (0.9).	50
3.9	Phase separation of emulsion prepared using crude extract from <i>E. scaber</i> .	51
3.10	Particle size of oil droplets stabilized by extracts from <i>E. scaber</i> . (a) D (0.5) and (b) D (0.9).	51
3.11	Phase separation of emulsion prepared using crude extract from <i>A. paniculata</i> .	52
3.12	Particle size of oil droplets stabilized by extracts from <i>A. paniculata</i> . (a) 3.12 D(0.5) and (b) D(0.9).	52

Part 2

3.1	Antimicrobial active plant extracts against various tested microorganisms at 50 mg/ml with ciprofloxacin (cip, 5 µg/disc) as positive control (placed at the center of each plate).	79-81
	(a) CL1 and CL3 against <i>B. subtilis</i> .	
	(b) CL3 against <i>E. coli</i> .	
	(c) CL2 against <i>P. aeruginosa</i> .	
	(d) CL1 and CL2 against <i>S. aureus</i> .	

- (e) TL1 and TL4 against *S. aureus*.
- (f) BU1, BU3 and BU4 against *S. aureus*.
- (g) BU3 against *E. coli*.
- (h) HB2 and HB3 against *B. subtilis*.

- 3.2 Determination of Minimal Bactericidal Concentrations (MBC) of extracts. 83
- (a) ET3 against *P. aeruginosa*,
 - (b) ET3 against *B. subtilis*.

LIST OF ABBREVIATIONS

AES	alcohol ether sulfate
ASM	American Society for Microbiology
BU1	ethanol-water (1:1) extract of Beluru (<i>E.spiralis</i>)
BU2	ethanol-water (9:1) extract of Beluru (<i>E.spiralis</i>)
BU3	chloroform-isopropanol (1:1) extract of Beluru (<i>E.spiralis</i>)
BU4	Water extract of Beluru (<i>E.spiralis</i>)
Cip	ciprofloxacin
CL1	ethanol-water (1:1) extract of Clove (<i>S.aromaticum</i>)
CL2	ethanol-water (9:1) extract of Clove (<i>S.aromaticum</i>)
CL3	chloroform-isopropanol (1:1) extract of Clove (<i>S.aromaticum</i>)
CL4	water extract of Clove (<i>S.aromaticum</i>)
Cmc	critical micelle concentration
DDW	double distilled water
D(0.1)	Diameter at which 10% of the volume falls below it
D(0.5)	Diameter at which 50% of the volume falls below it
D(0.9)	Diameter at which 90% of the volume falls below it
DMSO	Dimethyl sulfoxide
ET1	Ethanol-water 1:1
ET2	Ethanol-water 9:1
ET3	Chloroform-isopropanol 1:1
ET4	Water
FG1	ethanol-water (1:1) extract of Fenugreek (<i>T. foenum</i>)
FG2	ethanol-water (9:1) extract of Fenugreek (<i>T. foenum</i>)
FG3	chloroform-isopropanol (1:1) extract of Fenugreek (<i>T. foenum</i>)

FG4	water extract of Fenugreek (<i>T. foenum</i>)
HB1	ethanol-water (1:1) extract of Hempedu Bumi (<i>A. paniculata</i>)
HB2	ethanol-water (9:1) extract of Hempedu Bumi (<i>A. paniculata</i>)
HB3	chloroform-isopropanol (1:1) extract of Hempedu Bumi (<i>A. paniculata</i>)
HB4	water extract of Hempedu Bumi (<i>A. paniculata</i>)
HLB	Hydrophile-lipophile balance
LMW	Protein and low molecular weight
MBC	minimum bactericidal concentration
MIC	minimum inhibitory concentration
MH	Mueller-Hinton broth
O/W	water –in- oil emulsions
RDS	respiratory distress syndrome
SDS	sodium dodecyl sulfate
SO ₃	sulfur trioxide
SD	Standard deviation
TL1	ethanol-water (1:1) extract of Tapak Leman (<i>E. scaber</i>)
TL2	ethanol-water (9:1) extract of Tapak Leman (<i>E. scaber</i>)
TL3	chloroform-isopropanol (1:1) extract Tapak Leman (<i>E. scaber</i>)
TL4	Tapak Leman (<i>E. scaber</i>) emulsion from water extract
W/O	water –in- oil emulsions

LIST OF SYMBOLS

γ	Dyne/cm- unit of force of surface tension an interfacial tension
μ	Mu- unit of mass equal to 1/1,000,000 of a gram or milliliter. yield stress

PART ONE

INTERFACIAL ACTIVITY AND

EMULSION FORMATION

USING CRUDE EXTRACTS OF MEDICINAL

PLANTS AS EMULSIFIERS

CHAPTER 1

INTRODUCTION

Plants contain a variety of components such as, carbohydrates, lipids, amino acids, peptides, proteins, enzymes, phenolics, acetates, terpenoids, steroids, alkaloids and saponins. Some of these components may have surface activity due to their chemical structures and would be of great significance in the preparation of stable emulsion as well as having antimicrobial activities. Saponin for instance has surface activity which can be exploited for emulsion formation. The aim of this research is to screen for plant extracts from Malaysian medicinal plants with surface active properties and to use them for the preparation of stable pharmaceutical emulsions. It is also the intention of this work to determine the plant extracts that exhibit good antimicrobial properties.

1.1 PLANTS USED IN THIS STUDY

In our preliminary work (Hadi, Taher and Ahmad, 2008), 11 types of common medicinal plants were selected. The surface activities of the 50% ethanol-water crude extracts at various concentrations were determined. The crude extracts from (Beluru, Clove, Fenugreek, Hempedu Bumi and Tapak Leman) significantly reduced the surface tension of water (below 45 mN/m) at a concentration of 0.20%. Thus these plants were chosen for further studies.

1.1.1 Fenugreek (*Trigonella foenum -graecum*)

Trigonella foenum- graecum is a medicinal plant belonging to the legume family. It is widely grown in eastern countries and can be found in South East Asia including

Malaysia (Flammang, Cifone, Ereson and Stankowskci, 2004). *T. foenum-graecum* seeds are a rich source of protein and polysaccharides called galactomannans. The chemical components of the seeds include a large carbohydrate fraction (mucilaginous fiber, galactomannan), 20-30% proteins high in tryptophan and lysine, pyridine-type alkaloids, flavonoids, saponins, glycosides, vitamins, minerals, and volatile oils (Zohary and Hopf, 2000) as shown in Table 1.1. In a recent study, fenugreek seeds were experimentally shown to protect against cancers of the breast (Amin, Alkaabi, Al-Falasi and Daou, 2005). Ground fenugreek seeds are known for their health benefits with regard to reduction of cholesterol and sugar levels in the blood stream (Madar and Shomer, 1990).

Table 1.1
Chemical components of *T. foenum-graecum* seeds.

Component	Percent (%)
Dietary fiber	45.4
-Insoluble	-32.1
-Soluble	-13.3
Protein	36
Oil	6
Ash	3.2
Starch	1.6
Sugar	0.4

Sourced from Slinkard (2009).

Galactomannans are the most important components that are found in *T. foenum-graecum* seeds. These compounds have high water-binding capacity and the ability to form very viscous solutions at relatively low concentration (Ana, Fernandes, Mangrich, Sierakowski and Szpoganicz, 2001). Garti, Madar, Aserin and Sternheim in

1997, reported that the purified galactomannans from *T. foenum-graecum* reduced surface tension to values lower than guar gum, which is a galactomannan extracted from the guar bean. Furthermore, the interfacial activity was better than other galactomannans which lead to the formation of oil-in-water emulsions with small droplet size (2-3 μm) and long-term stability. The gum of *T. foenum-graecum* was found to adsorb (or ‘precipitate’) at the oil interface forming a relatively thick interfacial film. The emulsions prepared from its galactomannan were more stable than any equivalent emulsions stabilized by other gums. No flocculation was observed in emulsions stabilized with *T. foenum-graecum*. Galactomannans are widely used in foods as emulsifier agents.

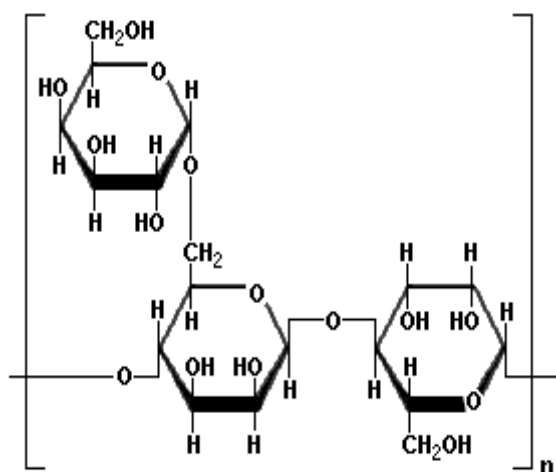


Figure 1.1: Structure of galactomannan

Solubility of a protein under varying conditions is one of its important functional properties because it greatly influences the application such as in emulsification (Kinsella, 1982). The emulsifying properties of *T. foenum-graecum* seeds are related to the processing procedure and to the protein composition. The emulsion capacity depends on the hydrophobic-lipophilic balance, which is affected by pH (Garti et al., 1997; El-Nasri and El-Tinay, 2007).

Omoloso and Vagi (2001), reported strong activity of *T. foenum-graecum* against 26 bacterial pathogens. It was also reported that the oil of *T. foenum-graecum* showed strong inhibition against *S. aureus*, *P. aeruginosa* and *A. niger*. The oil was effective even after only 24 h incubation against *S. aureus* and *P. aeruginosa* (Pritee, Rai, Deshmukh and Teixeira, 2007).

1.1.2 Clove (*Syzygium aromaticum*)

Syzygium aromaticum is a plant that has aromatic dry flower buds from a tree in the family Myrtaceae. It is used as a spice in cuisine all over the world. Cloves are harvested primarily in Zanzibar, Indonesia, Malaysia, India, Pakistan, and Sri Lanka. The compound responsible for the aroma is eugenol (Figure 1.2). Eugenol (C₁₀H₁₂O₂, 4-allyl-2-methoxy-phenol) is an allyl chain-substituted guaiacol which is a member of phenylpropanoids class of phytochemicals. It is the main component in the essential oil extracted from *S. aromaticum*, with a composition of 72-90%. Other important constituents include essential oils acetyl eugenol, beta-caryophylline and vanillin; crategolic acid; tannins, gallo tannic acid, methyl salicylate (painkiller); the flavanoids eugenin, kaempferol, rhamnetin, and eugenitin; triterpenoids like oleanolic acid, stigmasterol and campesterol; and several sesquiterpenes (Chaieb, Hajlaoui, Zmantar, Kahla-Nakbi, Rouabhia, Mahdouani and Bakhrouf, 2007).

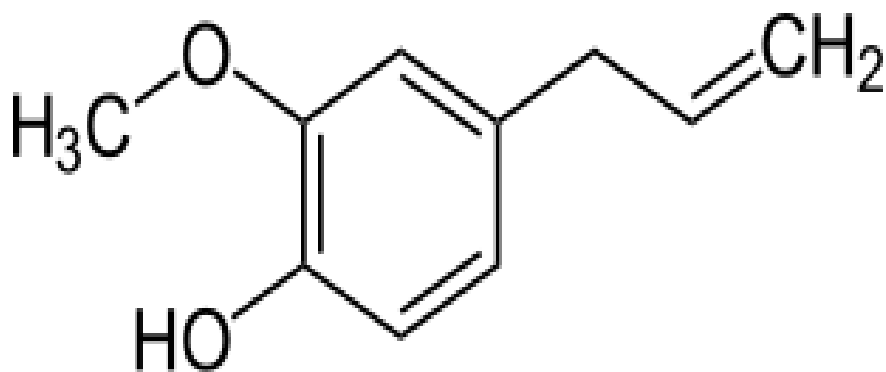


Figure 1.2: Structure of eugenol

Liu reported the emulsification of clove oil using phosphate buffer as the continuous phase and sodium dodecyl sulfate (SDS) as the surfactant (Liu, Nakajima, Nabetani, Yi Xu, Ichikawa and Sano, 2000). The average diameter of oil the droplets was about 20 μm with a narrow size distribution. The stability characteristics of the dispersed oil droplets were investigated by an optical microscope and kinetic light scattering method.

The essential oil extracted is used as a topical application to relieve pain and promote healing in herbal medicine with other uses in fragrance and flavoring industries. Because of the chemical components and biological effects of clove essential oil, it is used as medication for antimicrobial, antioxidant, antifungal and antiviral activity, anti-inflammatory, cytotoxic and anesthetic properties (Chaieb et al., 2007). The antimicrobial activity of the essential oils from *S. aromaticum* was tested; it possessed significant antimicrobial effects against many kinds of microorganisms tested.