

Table of Contents

Abstract	i-iii	
Declaration's declaration	iv	
Certificate of the Supervisors	v	
Acknowledgement	vi-vii	
Table of Contents	viii-xiii	
List of Figures	xiv-xviii	
List of Tables	xix-xx	
List of Abbreviations	xxi-xxiii	
Chapter 1 Introduction		1
1. Introduction		1
1.1. Food as medicine		2
1.2. Plant secondary metabolites		2
1.3. Traditional medicine and a brief introduction of garlic mustard oil macerate		2
1.4. Garlic (<i>Allium sativum</i>)		3
1.5. Black mustard (<i>Brassica nigra</i>) and its biochemical composition		3
1.6. Biochemical and phytochemistry of garlic macerate in vegetable oils		4
1.7. Optimization of the traditional medicine preparation		5
1.8. Traditional use of garlic mustard oil macerate and its bioactivity		5
1.9. Gap in study		8
1.10. Objectives of the present research		8
1.11. References		9-14
Chapter 2 Review of Literature		15
2. Review of Literature		16
2.1. Physicochemical and photochemical analysis of garlic macerate in various oil		17
2.2. Optimization of garlic macerate in various vegetable oils		20
2.3. Bioactive properties of thiosulfinate compounds present in garlic oil macerate		21
2.4. References		24-27
Chapter 3 Materials and Methods		28
3. Materials and methods		28
3.1. Collection of mustard (<i>Brassica nigra</i>) seed and garlic (<i>Allium sativum</i>)		29
3.2. Biochemical composition of mustard (<i>Brassica nigra</i>) Seeds and Garlic (<i>Allium sativum</i>)		29
3.2.1. Moisture content		29
3.2.2. Percentage of oil in mustard seeds		29
3.2.3. Ash content		29
3.2.4. Total Carbohydrate		30
3.2.5. Crude Fibre content		30
3.2.6. Crude Fat		31
3.2.7. Crude protein		31
3.3. Physicochemical examination and phytochemical analysis of oil from GMM		31
3.3.1. Preparation of Garlic mustard oil macerate (GMM)		31
3.3.2. Preparation of garlic toluene extract (GTE)		32
3.3.3. Physicochemical analysis of GMM oil		32
3.3.3.1. Chemical properties		32
3.3.3.1.1. Free fatty acid		32
3.3.3.1.2. Peroxide value		32
3.3.3.1.3. Acid value		33
3.3.3.1.4. Iodine value		33
3.3.3.1.5. Saponification value		34

3.3.3.2.	Physical properties	34
3.3.3.2.1.	Determination of the specific gravity of mustard oil and GMM	34
3.3.3.2.2.	Determination of viscosity of mustard oil and GMM	35
3.3.3.2.3.	Determine the refractive index of mustard oil and GMM	35
3.3.4.	Phytochemical analysis	35
3.3.4.1.	FTIR analysis	35
3.3.4.2.	Total polyphenol content	35
3.3.4.3.	Antioxidant activity	35
3.3.4.4.	Thin Layer Chromatography	36
3.3.4.5.	High-Performance Liquid Chromatography	36
3.3.4.6.	Liquid chromatography-mass spectrometry (LCMS) analysis	36
3.3.4.7.	Gas chromatography-mass spectrometry (GCMS) analysis	37
3.4.	Optimization of garlic mustard oil macerate	37
3.4.1.	Optimization of garlic mustard oil macerate using response surface methodology	37
3.4.1.1.	Experimental design and process optimization	37
3.4.1.2.	High-Performance Liquid Chromatography activity analysis of experimental designs	39
3.4.2.	Optimization of GMM based on antibacterial activity and antifungal activity	40
3.4.2.1.	Quantification of the amount of GMM required for antibacterial and antifungal activity during vapour diffusion assay	40
3.4.2.2.	Optimization of GMM based on antibacterial activity against <i>S. aureus</i>	41
3.4.2.2.1.	Antibacterial activity by vapour diffusion method	41
3.4.2.3.	Optimization of GMM based on antifungal activity against <i>Candida albicans</i> MTCC 410	41
3.4.2.3.1.	Antifungal activity by vapour diffusion method	41
3.4.2.3.2.	Antifungal activity by agar diffusion method	41
3.4.2.4.	Preliminary preparation of GMM	42
3.4.2.5.	Preparation of GMM based on the ratio of garlic and mustard oil	42
3.4.2.6.	Preparation of GMM based on time of heating of macerate at 160°C	42
3.4.2.7.	Preparation of GMM based on time of heating of macerate at 80°C	43
3.5.	Bioactivity of optimized GMM	43
3.5.1.	Antibacterial activity	43
3.5.1.1.	Bacterial culture	43
3.5.1.2.	Agar diffusion	43
3.5.1.3.	Vapour diffusion	43
3.5.1.4.	Minimum inhibitory concentration and minimum bactericidal concentration	44
3.5.1.5.	Gram staining	44
3.5.1.6.	Staphyloxanthin inhibition by GMM against <i>S. aureus</i>	44
3.5.1.6.1.	Inhibition percentage of staphyloxanthin	45
3.5.1.6.2.	Fourier-transform infrared spectroscopy (FTIR) analysis	45
3.5.1.6.3.	Scanning electron micrography (SEM) analysis	46
3.5.1.6.4.	Molecular docking analysis of GMM volatile compounds with Dehydroxysqualene synthase (CrtM) protein	46
3.5.2.	Antibiofilm analysis	46
3.5.2.1.	Screening of bacteria producing biofilm	46
3.5.2.2.	Antibiofilm activity against <i>S. aureus</i>	47
3.5.2.2.1.	Agar diffusion assay	47
3.5.2.2.2.	Vapour diffusion assay	47
3.5.2.2.3.	Ring-biofilm inhibition assay	47

3.5.2.3.	Antibiofilm activity against <i>Pseudomonas aeruginosa</i> MTCC 2297	47
3.5.2.3.1.	Vapour diffusion assay	47
3.5.2.3.2.	Microscopic analysis	47
3.5.2.3.3.	Estimation of total carbohydrate in EPS	48
3.5.2.3.4.	Pyocyanin inhibition	48
3.5.2.4.	Violacein inhibition assay in <i>Chromobacterium violaceum</i> MTCC 12472	48
3.5.2.4.1.	Vapour diffusion assay	48
3.5.2.4.2.	Violacein inhibition	48
3.5.3.	Antifungal activity	49
3.5.3.1.	Vapour diffusion assay	49
3.5.3.2.	Agar diffusion assay	49
3.5.3.3.	Minimum inhibitory concentration and minimum fungicidal concentration determination by agar diffusion assay	49
3.5.3.4.	Lactophenol blue staining	50
3.5.3.5.	Poison food assay	50
3.5.3.6.	Scanning electron microscopy	50
3.5.3.7.	Molecular docking analysis of GMM volatile compounds with N-myristoyltransferase (<i>nmt</i>) protein	51
3.5.4.	Cell viability assay against HEK293 normal cell line, THP-1 normal cell line, and MCF7 breast cancer cell line	51
3.5.4.1.	GMM sample preparation for cell culture	51
3.5.4.2.	Cell culture and cell viability assay	51
3.5.5.	Anti-inflammatory activity against THP-1 cell line	52
3.5.5.1.	Treatment for studying the effect of GMM/ MO on gene expression	52
3.5.5.2.	Preparation of cDNA synthesis (first-strand)	52
3.5.5.3.	Semi-quantitative PCR	52
3.5.5.4.	ADME (Absorption, Distribution, Metabolism, and Excretion) and drug-likeness analysis	53
3.5.5.5.	Molecular docking analysis of GMM volatile compounds with Cox2, IL1 β , IL6, TNF α and IL-8	53
3.5.6.	Transdermal activity	54
3.5.6.1.	Preparation of the eggshell membrane	54
3.5.6.2.	In-vitro drug release	55
3.5.7.	Sensory acceptability test	55
3.6.	References	56-59
Chapter 4	Results	60
4.	Results	60
4.1.	Biochemical composition of mustard (<i>Brassica nigra</i>) seeds and garlic (<i>Allium sativum</i>)	61
4.1.1.	Moisture content	61
4.1.2.	Ash content	61
4.1.3.	Total carbohydrate	61
4.1.4.	Crude fibre content	61
4.1.5.	Crude fat	61
4.1.6.	Crude protein	61
4.2.	Physicochemical examination and phytochemical analysis of oil from GMM	62
4.2.1.	Chemical properties	62
4.2.1.1.	Free fatty acid	62
4.2.1.2.	Peroxide value	62
4.2.1.3.	Acid value	63

4.2.1.4.	Iodine value	64
4.2.1.5.	Saponification value	64
4.2.2.	Physical properties	65
4.2.2.1.	Density and specific gravity	65
4.2.2.2.	Viscosity	65
4.2.2.3.	Refractive index	65
4.2.3.	Phytochemical analysis	65
4.2.3.1.	FTIR analysis	65
4.2.3.2.	Total polyphenol content	67
4.2.3.3.	Antioxidant activity	67
4.2.3.4.	TLC analysis of raw garlic, MO, and GMM	69
4.2.3.5.	High-performance liquid chromatography analysis of GTE, MO, and GMM	69
4.2.3.6.	Liquid chromatography-mass spectrometry analysis of GTE, MO, and GMM	70
4.2.3.6.1.	LCMS analysis of GTE	70
4.2.3.6.2.	LCMS analysis of MO	72
4.2.3.6.3.	LCMS analysis of GMM	74
4.2.3.7.	Gas chromatography-mass spectrometry analysis	78
4.3.	Optimization of garlic mustard oil macerate	80
4.3.1.	Optimization of garlic mustard oil macerate using response surface methodology	80
4.3.2.	Quantification of the amount of GMM oil required for antibacterial and antifungal activity during vapour diffusion assay	83
4.3.3.	Optimization of GMM based on antibacterial activity against <i>S. aureus</i>	86
4.3.3.1.	Antibacterial activity by vapour diffusion method	86
4.3.3.1.1.	GMM based on the ratio of garlic and mustard oil	86
4.3.3.1.2.	GMM based on time of heating of macerate at 80°C	87
4.3.4.	Optimization of garlic mustard oil macerate based on antifungal activity	88
4.3.4.1.	Antifungal activity by agar diffusion method	88
4.3.4.1.1.	GMM based on different preparation temperature	88
4.3.4.1.2.	GMM based on the time of heating	89
4.3.4.1.3.	GMM based on the ratio of garlic and mustard oil	90
4.3.4.2.	Antifungal activity by vapour diffusion method	91
4.3.4.2.1.	GMM based on the ratio of garlic and mustard oil	91
4.3.4.2.2.	GMM based on time of heating of macerate at 160°C	92
4.3.4.2.3.	GMM based on time of heating of macerate at 80°C	93
4.3.5.	Optimization of garlic mustard oil macerate based on DPPH scavenging activity	95
4.3.5.1.	GMM based on the ratio of garlic and mustard oil	95
4.3.5.2.	GMM based on the temperature of heating	95
4.3.5.3.	GMM based on incubation time	96
4.4.	Bioactivity of optimized garlic mustard oil macerate	96
4.4.1.	Antibacterial activity	96
4.4.1.1.	Vapour diffusion assay	96
4.4.1.2.	Minimum inhibitory concentration and minimum bactericidal concentration using agar diffusion assay	98
4.4.1.3.	Gram staining	99
4.4.1.4.	Staphyloxanthin inhibition by GMM against <i>S. aureus</i>	100
4.4.1.4.1.	Inhibition percentage of staphyloxanthin	101
4.4.1.4.2.	Fourier-transform infrared spectroscopy (FTIR) analysis	102
4.4.1.4.3.	Scanning electron microscopy (SEM) analysis	102
4.4.1.4.4.	Molecular docking	103

4.4.2.	Antibiofilm analysis	106
4.4.2.1.	Screening of bacteria producing biofilm	106
4.4.2.2.	Antibiofilm assay against <i>S. aureus</i>	106
4.4.2.2.1.	Agar diffusion assay	106
4.4.2.2.2.	Vapour diffusion assay	107
4.4.2.2.3.	Ring Biofilm inhibition assay	107
4.4.2.3.	Antibiofilm activity against <i>Pseudomonas aeruginosa</i> MTCC 2297	108
4.4.2.3.1.	Vapour diffusion assay	108
4.4.2.3.2.	Microscopic assay	108
4.4.2.3.3.	Estimation of total carbohydrate in EPS	109
4.4.2.3.4.	Quantification of pyocyanin	110
4.4.2.4.	Violacein inhibition assay in <i>Chromobacterium violaceum</i> MTCC 12472	110
4.4.2.4.1.	Vapour diffusion assay	110
4.4.2.4.2.	Violacein inhibition	111
4.4.3.	Antifungal activity	111
4.4.3.1.	Vapour diffusion assay	111
4.4.3.2.	MIC and MBC determination by agar diffusion assay	112
4.4.3.3.	Lactophenol blue staining	113
4.4.3.4.	Poison food assay	113
4.4.3.5.	Scanning electron microscopy	115
4.4.3.6.	Molecular docking	115
4.4.4.	Cell viability assay against HEK293 normal cell line, THP-1 cell line, and MCF7 breast cancer cell line	119
4.4.5.	Anti-inflammatory activity	121
4.4.5.1.	Semi-quantitative PCR	121
4.4.5.2.	ADME (Absorption, Distribution, Metabolism, and Excretion) and drug-likeness analysis	122
4.4.5.3.	Molecular docking analysis of GMM volatile compounds with Cox2, IL1 β , IL6, TNF α and IL-8	125
4.4.5.3.1.	Cox2 (PDB ID: 4cox)	125
4.4.5.3.2.	IL-1 β (PDB ID: 1itb)	126
4.4.5.3.3.	IL-6 (PDB ID: 1n26)	127
4.4.5.3.4.	TNF- α (PDB ID: 2az5)	128
4.4.5.3.5.	IL-8 (PDB ID: 4xdx)	129
4.4.6.	Transdermal activity	130
4.4.7.	Sensory acceptability test	135
4.5.	Reference	135
Chapter 5	Discussion	136
5.	Discussion	136
5.1.	Biochemical composition of mustard (<i>Brassica nigra</i>) seeds and garlic (<i>Allium sativum</i>)	137
5.2.	Physicochemical examination and phytochemical analysis of oil from GMM	137
5.2.1.	Chemical properties	137
5.2.2.	Physical properties	138
5.2.3.	Phytochemical analysis	138
5.3.	Optimization of garlic mustard oil macerate	141
5.4.	Bioactivity of optimized garlic mustard oil macerate	143
5.4.1.	Antibacterial activity	143
5.4.2.	Antibiofilm and anti-quorum sensing analysis	144
5.4.3.	Antifungal activity	146

5.4.4.	Cell viability assay against HEK293 normal cell line, THP-1 cell line, and MCF7 breast cancer cell line	148
5.4.5.	Anti-inflammatory activity	149
5.4.6.	ADME and drug-likeness analysis	150
5.4.7.	Transdermal activity	151
5.4.8.	Sensory acceptability test	152
5.5.	References	152-
		160
Chapter 6	Conclusion and Future Perspectives	161
6.	Conclusion and Future Perspectives	161
6.1.	Conclusion	162
6.2.	Future Prospects	164
7.	Publications and conferences	166-
		171