DEVELOPMENT OF PROCESS FOR EXTRACTION OF FLORAL FRAGRANCES BY SUBCRITICAL CARBON DIOXIDE

by

PRASANTA KUMAR ROUT

Centre for Rural Development and Technology

Submitted

in fulfillment of the requirement of the degree of

DOCTOR OF PHILOSOPHY

to the

INDIAN INSTITUTE OF TECHNOLOGY, DELHI

HAUZ KHAS, NEW DELHI – 110016, INDIA

SEPTEMBER 2008

To my Mother

CERTIFICATE

This is to certify that the thesis entitled, "**DEVELOPMENT OF PROCESS FOR EXTRACTION OF FLORAL FRAGRANCES BY SUBCRITICAL CARBON DIOXIDE**" being submitted by **Mr. Prasanta Kumar Rout** to the Indian Institute of Technology, Delhi for the award of Doctor of Philosophy is a record of bonafide research work carried out by him under my guidance and supervision in conformity with the rules and regulations of Indian Institute of Technology, Delhi

The research report and results presented in this thesis have not been submitted, in part or in full, to any other university of institute for the award of any degree or diploma.

SNaik

(S. N. Naik)
Professor
Centre for Rural Development and Technology
Indian Institute of Technology, Delhi
New Delhi-110016, India

I express gratitude to Council of Scientific and Industrial Research (CSIR), New Delhi, India for providing me financial assistance in the form of Senior Research Fellow.

Lastly, I am ever indebted to my mother whose blessings and prayers boosted my inner strength to reach me upto this level. The affection, moral support and encouragement of my Brother, Sister and Sister-in-law throughout my academic pursuits is gratefully acknowledged.

PK Rout

(Prasanta Kumar Rout)

The essential oil obtained by hydrodistillation, pentane or hexane extracts (concrete), fractionation of concrete with methanol to get absolute and subcritical CO_2 extract of flowers and concrete were analysed by GC-FID and GC/MS. The qualitative analysis of picked and live (flowers attached to the plant) flowers were carried out by HS-SPME with GC and GC/MS.

The yield of the essential oil is very less and it does not represent the true components present in the flowers. The limitations of the process are loss of the water-soluble essential oil components in the distillation water. In addition, prolonged heating of the essential oil in the presence of water results in various chemical changes (hydrolysis, molecular rearrangement, recimisation and degradation). The cohobated oil often exhibits an altered and inferior organoleptic profile. So the process is not suitable for extraction of fragrance from delicate flowers.

The process of production of concrete by non-polar hydrocarbon solvents viz. pentane or hexane is a better route for extraction of low yield and precious floral fragrances. But the solvent extracts carry waxy components co-extracted along with the desirable components. The fractionation of concrete with methanol can separate a part of the waxy components,and the absolute still carried fair amounts of fatty components. Though absolute is a better form of the floral extract but there is chance of loss of top note fragrance components with solvent at the solvent recovery step and also the product is not free from solvent residues.

The subcritical CO_2 extraction process overcomes the above mentioned drawbacks of solvent extraction. Subcritical CO_2 extract of flowers has given superior products with improved yield of desirable components with minimum amount of waxy components. But bulk volume of the flowers is large in comparison to the yield the floral fragrance. So, for commercial scale production, very large subcritical CO_2 extraction unit is needed.

Alternatively fractionation of floral concrete by subcritical CO_2 is comparable with subcritical CO_2 extract of flowers. The isolate is enriched with the desirable components and free from solvent residues. The optimum time period for extraction of desirable components at 20°C temperature and 65 bar pressure is 3 hours. It was therefore decided to fractionate the concrete in two stages of extractions with 90 minute duration in each extraction. The subcritical CO_2 fraction obtained in the first stage was organoleptically superior and enriched with desirable components and contained very less amounts of waxy components in comparison to subcritical CO_2 fraction obtained in the second stage.

Direct subcritical CO_2 extraction gave isolate which is free from solvents and enriched with desired components but due to the reasons mentioned already, it does not make an attractive proposal for commercial process, but subcritical CO_2 fractionation of floral concrete is an attractive proposal.

The compound Manool has not been reported in Murraya paniculata Linn. floral extract so far and we report isolation of this compound for the first time. Similarly Quinoline-4-carbonitrile has been isolated from Quisqualis indica Dent. floral extract and this new compound has been reported for first the time as a natural compound.

ACKNOWLEDGEMENT

Gratitude can seldom be expressed in words.

Beginning with the formulation of research problem, till date, I have been especially privileged one to receive guidance from my supervisor Dr. S. N. Naik whose academic excellence and constant encouragement steered me through the work all the way and all the time. I would extend the opportunity to express my deep sense of gratitude for his motivational urge, valuable analysis, criticism and personal affection which installed in me immense confidence to continue my research right from the beginning of my research work till the accomplishment of the goal.

I am sincerely thankful to Prof. R.C. Maheshwari, Professor CRDT, for guidance and especially gave an opportunity to work with him in subcritical CO_2 extraction of different flowers and spices. I am indebted his contribution and valuable suggestion through out my Ph.D work.

I am extremely grateful to Dr. Y.R. Rao, Ex-Scientist and Head, Natural Products Department, Regional Research Laboratory (CSIR), Bhubaneswar for the valuable inputs and suggestions through out my research period. He is the source of inspiration of my research work and my association with him during project period became the turning point of my career that motivated me for research carrier. His affection and financial assistance gave me strength to work against all odds.

I thankful to Prof. A. K. Gupta, Dept. of Chemical Engineering and Dr. A. Sree, Scientist-F, Dr. S. Sahoo, Scientist-F, Dr. A. Nayak, Scientist and Head, Natural Products Department, Regional Research Laboratory (CSIR), Bhubaneswar for their helpful discussion, suggestions and encouragement.

I am indebted to my friends Dr. Meher, Ms. Deeptanjali and Mr. Kabi for the help as well as valuable discussions and suggestions provided during my research. I acknowledge the co-operations from Jadeja, Asheesh, Rama, Ganesh and Vidyaswamy during my thesis work.

I am grateful to Prof. Santosh Satya, Chairman of my SRC, Prof. P. L. Dhar HOD, CRDT, Prof. Rajendra Prasad, Prof. P. Vasudaven, Dr. Satyawati Sharma, Dr. V. K. Vijay, Dr. A. Malik and Dr. V. M. Chariar for laboratory facility and encouragement and office staff of the Centre for providing laboratory facilities and timely requirements for my research work.

I feel pleased to acknowledge the love, affection and support received from Mr. and Mrs. Madhumita Patel, Narendra, Ajaya Dash, Gajada, Malaya, Lalit joshi, Srikant Pradhan, B. Bahinipati, Subhalaxmi, Mamun and Mrs. Jyoti Manjari Naik.

When one owes so many, it is almost impossible and even invidious to single out names. However, I am indebted to my friends Lalit, Ashwani, Ramu, Parag, R. Katiyar, Kishore, Mohini, Pratibha, Monica and Mewalal. Also to my friends Satya, Naba, Saroj, Kamalakant and Srikant, Chemistry Department, Kapil, CES, Praveen, Civil, Gyani, IDDC, for their constant encouragement and wholehearted support without which it would have been difficult to finish this task.

ABSTRACT

Floriculture is an age-old farm activity with immense potential for generating remunerative self-employment among small and marginal farmers. India is bestowed with diverse agro-climatic zones, which enables it to produce large varieties of flowers like rose, jasmine, marigold, champa, kewda, tuberose etc. They are commercially cultivated in India for extraction of floral fragrances. These resources can be utilized to produce value added floral fragrances/ essential oils to meet the Indian and International demand. The world's total production of essential oils is estimated at about 1.0-1.1 lakh tons and India stands at third position with a share of 18-20% and second position in processed value added products. The traditional cottage essential oil industry of India used several natural plant raw materials like leaves, flowers, fruits, wood, roots, resins and gums. The demand of essential oils and extracts are increasing day by day. The floral extracts are not being produced on large scale in spite of vast potential in this sector. Hence there is a great scope for commercial cultivation of flower crops for production of floral fragrance to meet the growing demand.

Flowers produce different types of fragrances probably as a biological function to attract insects for helping pollination. Floral fragrances are used in several applications from high-grade perfumery to flavouring agents and even as odour masking agents. Several formulations based on composition of these floral oils are used in industrial applications such as soaps, detergents, scented tobacco, incense, toilet and hygienic products and their demand runs into several hundreds to tons.

Scented flowers are the natural sources for extraction of perfumes and flavours. These extracts may be in the form of concrete, absolute, attar, essential oil etc. Experiments were hence carried out to assess the quality and quantity of the extracts. Supercritical CO_2 *is an*

advanced process for extraction of floral fragrances. The subcritical CO_2 extraction (Liquid CO_2 extraction) has certain advantages over supercritical CO_2 extraction particularly when dealing with flavour and fragrance compounds that have medium molecular mass (<300) and with low polarity. This includes compounds such as oxygenated and non oxygenated mono and sesquiterpene compounds found in floral volatiles.

In the course of our studies, we have come across wide variation in the composition of different extracts due to variability of extraction or formation of artifacts etc. With the objective of determining the composition of fragrance emanated by flowers as such, we have carried out Head-space SPME analysis and results are quite interesting.

HS-SPME is a solvent free sample preparation technique in which a fused silica fiber coated with a polymeric organic liquid is introduced into the headspace of the flowers, placed in a closed container. The volatile compounds emanated by flowers get adsorbed onto the coating and concentrated for desorption and analysis. The analytes were concentrated enough to be detected by GC and GC/MS. The adsorbed materials on the fiber are thermally desorbed into the hot zone of the injection port and carried into the column for separation and analysis.

The flowers selected for the present study are Jasmine (Jasminum sambac Linn), Champa (Michelia champaca Linn), Kewda (Pandanus fascicularis Lam), Murraya (Murraya paniculata Linn) and Rangoon creeper or Madhumalati (Quisqualis indica Dent). The flowers such as Jasminum sambac, Michelia champaca and Pandanus fascicularis are cultivated for decoration, worship and extraction of fragrance, where as Murraya paniculata and Quisqualis indica are cultivated for gardening purpose *only. Presently we have studied the different methods of extraction of floral fragrances and compared the chemical compositions of the extracts.*

CONTENTS

Acknowledgement i

Abstract iv

List of Figures viii

List of Tables xii

List of Schemes xv

Notations xvi

CHAPTER-I: INTRODUCTION AND LITERATURE REVIEW **1-24**

1.1 Background 1

1.2 Flowers selected for present study 2

1.2.1 Jasmine (*Jasminum sambac* Linn) 2

1.2.1.1 General characteristics of the plant 2

1.2.1.2 Chemical composition of floral extracts 3

1.2.2 Champa (*Michelia champaca* Linn) 5

1.2.2.1 General characteristics of the plant 5

1.2.2.2 Chemical composition of floral extracts 6

1.2.3 Kewda (*Pandanus fascicularis* Lam) 7

1.2.3.1 General characteristics of the plant 7

1.2.3.2 Chemical composition of floral extracts 8

1.2.4 Orange Jessamine (*Murraya paniculata* Linn) 9

1.2.4.1 General characteristics of the plant 9

1.2.4.2 Chemical composition of floral extracts 10

1.2.5 Rangoon creeper (*Quisqualis indica* Dent) 11

1.2.5.1 General characteristics of the plant 11

1.2.5.2 Chemical composition of floral extracts 12

1.3 Existing methods of extraction processes for floral materials 12

1.3.1 Hydrodistillation 13

1.3.1.1 Decanted oil 13

1.3.1.2 Cohobated oil 14

1.3.1.3 Advantages and Drawbacks 14

1.3.2 Extraction with volatile solvents 15

1.3.2.1 Concrete 15

1.3.2.2 Absolute 15
1.3.2.3 Advantages and Drawbacks 16
1.4 Superior methods of extraction 16
1.4.1 Supercritical CO_2 extraction 17
1.4.2 Subcritical CO_2 extraction 18
1.4.2.1 Advantages 18
1.5. Previous work done and gaps in knowledge 19
1.6 Need for analysis of fragrance emanated from flowers 19
1.7 Objective of the present study 20
1.8 Analysis of flower fragrances by HS-SPME with GC and GC/MS 21
1.8.1 Headspace analysis techniques 21
1.8.1.1 Direct headspace injection 21
1.8.1.2 Purge and Trap (P & T) 22
1.9.1.3 Solid Phase Extraction (SPE) 22
1.8.1.4 Solid Phase Microextraction (SPME) 22
1.8.1.4.1 The SPME device 23
1.8.2 Headspace –Solid Phase Microextraction (HS-SPME) 24

CHAPTER-II: MATERIALS AND METHODS **25-37**
2.1 Flowers selected for experiment 25
2.2 Solvent extraction 26
2.2.1 Concrete 26
2.2.2 Absolute 27
2.2.3 Wax 27
2.3 Hydrodistillation 27
2.3.1 Hydrodistillation of flowers 28
2.3.2 Hydrodistillation of concrete 28
2.4 Subcritical CO_2 extraction 28
2.4.1 Subcritical CO_2 extraction of flowers 29
2.4.2 Subcritical CO_2 fractionation of concrete 29
2.5 Analysis of extracts 30
2.5.1 Qualitative analysis by GC 30
2.5.2 Identification of components (GC/MS analysis) 31

2.5.3 Relative Retention indices (Kovat indices) calculation 31
2.5.4 Identification of major compounds in GC by co-elution 32
2.5.5 Quantitative analysis 32
2.5.6 Isolation of compounds 32
2.5.6.1 Column chromatography 32
2.5.6.2 Thin layer chromatography (TLC) 32
2.5.6.3 Preparative-thin layer chromatography (PTLC) 33
2.5.7 Identification of compounds 33
2.5.7.1 Melting point 33
2.5.7.2 Elemental analysis 33
2.5.7.3 IR spectroscopy 33
2.5.7.4 Mass spectrometry 33
2.5.7.5 Nuclear magnetic resonance (NMR) spectroscopy 33
2.5.8 Synthesis of the compound 34
2.6 Headspace-Solid phase microextraction (HS-SPME) 34
2.6.1 PDMS fiber 34
2.6.2 Equilibrium time for adsorption of volatiles over PDMS fiber 35
2.6.3 Live flowers (flowers attached to the branch) analysis 35
2.6.4 Picked flowers analysis 36
2.6.5 GC and GC/MS analysis of adsorbed volatiles 37

CHAPTER-III: EVALUATION OF ESSENTIAL OILS OF FLOWERS AND VOLATILES OF CONCRETES OBTAINED BY HYDRODISTILLATION **38-79**

3.1 Essential oils and volatiles from concrete of *Jasminum sambac* Linn 38
3.1.1 Essential oil from flowers 38
3.1.2 Volatiles from concrete 39
3.1.3 Analysis of essential oils and volatiles from concrete 39
3.1.4 Results and Discussion 48
3.2 Essential oil and volatiles from concrete of *Michelia champaca* Linn 49
3.2.1 Essential oil from flowers 49
3.2.2 Volatiles from concrete 49
3.2.3 Analysis of essential oil and volatiles from concrete 49

	3.2.4 Results and Discussion	57
3.3	Essential oils and volatiles from concrete of *Pandanus fascicularis* Lam	57
	3.3.1 Essential oil from flowers	58
	3.3.2 Volatiles from concrete	58
	3.3.3 Analysis of essential oils and volatiles from concrete	58
	3.3.4 Results and Discussion	63
3.4	Essential oil and volatiles from concrete of *Murraya paniculata* Linn	63
	3.4.1 Essential oil from flowers	63
	3.4.2 Volatiles from concrete	63
	3.4.3 Analysis of essential oil and volatiles from concrete	64
	3.4.4 Results and Discussion	71
3.5	Essential oil and volatiles from concrete of *Quisqualis indica* Dent	71
	3.5.1 Essential oil from flowers	71
	3.5.2 Volatiles from concrete	72
	3.5.3 Analysis of essential oil and volatiles from concrete	72
	3.5.4 Results and Discussion	78

CHAPTER-IV: EVALUATION OF EXTRACTS OBTAINED BY SOLVENT EXTRACTION **80-123**

4.1	*Jasminum sambac* Linn	80
	4.1.1 Concrete	80
	4.1.2 Absolute	80
	4.1.3 Wax	80
	4.1.4 Analysis of the concrete, absolute and wax	80
	4.1.4.1 GC and GC/MS analysis	80
	4.1.4.2 GC co-elution with pure compounds	81
	4.1.4.3 Determination of volatile matter in the concrete and absolute	81
	4.1.5 Results and Discussion	84
4.2	*Michelia champaca* Linn	85
	4.2.1 Concrete	85
	4.2.2 Absolute	85
	4.2.3 Wax	85
	4.2.4 Analysis of the concrete, absolute and wax	86

4.2.4.1 GC and GC/MS analysis 86
4.2.4.2 GC co-elution with pure compounds 86
4.2.4.3 Determination of volatile matter in the concrete and absolute 86
4.2.5 Results and Discussion 88
4.3 *Pandanus fascicularis* Lam 89
4.3.1 Concrete 89
4.3.2 Absolute 89
4.3.3 Wax 90
4.3.4 Analysis of the concrete, absolute and wax 90
4.3.4.1 GC and GC/MS analysis 90
4.3.4.2 GC co-elution with pure compounds 90
4.3.4.3 Determination of volatile matter in the concrete and absolute 90
4.3.5 Results and Discussion 92
4.4 *Murraya paniculata* Linn 93
4.4.1 Concrete 94
4.4.2 Absolute 94
4.4.3 Wax 94
4.4.4 Analysis of the concrete, absolute and wax 94
4.4.4.1 GC and GC/MS analysis 94
4.4.4.2 GC co-elution of major compounds 95
4.4.4.3 Determination of volatile matter in the concrete and absolute 95
4.4.5 Fractionation of concrete 97
4.4.5.1 Column chromatographic separation of concrete 98
4.4.5.2 Preparative- thin layer chromatography 98
4.4.5.3 Thin layer chromatography 98
4.4.5.4 Analysis of the fraction by GC and GC/MS 99
4.4.5.5 Isolation of Manool 101
4.4.6 Identification of Manool 101
4.4.6.1Nature and melting point of Manool 101
4.4.6.2 Elemental analysis 102
4.4.6.3 IR spectroscopy 102
4.4.6.4 Mass spectrum 103
4.4.6.5 Nuclear magnetic resonance spectroscopy 104

4.4.6.5.1 ^{1}H-NMR spectrum 104
4.4.6.5.2 ^{13}C-NMR spectrum 105
4.4.6.6 Interpretation of spectroscopic data for Manool 105
4.4.7 Results and Discussion 106
4.5 *Quisqualis indica* Dent 107
4.5.1 Concrete 107
4.5.2 Absolute 107
4.5.3 Wax 107
4.5.4 Analysis of the concrete, absolute and wax 107
4.5.4.1 GC and GC/MS analysis 107
4.5.4.2 GC co-elution with pure compounds 108
4.5.4.3 Determination of volatile matter in the concrete and absolute 108
4.5.5 Isolation of the unknown compound 110
4.5.5.1 Column chromatographic separation of concrete 111
4.5.5.2 Preparative thin layer chromatography 111
4.5.5.3 Thin layer chromatography 111
4.5.6 Identification of the unknown compound 112
4.5.6.1 Elemental analysis 112
4.5.6.2 IR spectrum 112
4.5.6.3 Mass spectrum 113
4.5.6.4 Nuclear magnetic resonance spectroscopy 114
4.5.6.4.1 ^{1}H NMR spectrum 114
4.5.6.4.2 ^{13}C NMR spectrum 115
4.5.6.4.3 ^{1}H-^{1}H COSY spectrum 116
4.5.6.4.4 ^{1}H-^{13}C HMQC spectrum 117
4.5.6.5 Interpretation of spectroscopic data and identification of compound 117
4.5.6.6 ^{1}H-NMR of quinoline-2-carbonitrile 119
4.5.6.7 ^{13}C-NMR of quinoline-2-carbonitrile 120
4.5.6.8 Synthesis of the Quinoline-4-carbonitrile 121
4.5.7 Results and Discussion 122

CHAPTER-V: SUBCRITICAL CO_2 EXTRACTION **124-160**

5.1 *Jasminum sambac* Linn 124
5.1.1 Subcritical CO_2 extraction of flowers 126
5.1.2 Subcritical CO_2 fractions of concrete 126
5.1.2.1 Subcritical CO_2 fraction, first stage 127
5.1.2.2 Subcritical CO_2 fraction, second stage 127
5.1.3 Analysis of the extracts 127
5.1.3.1 GC and GC/MS analysis 127
5.1.3.2 Determination of volatile matter in the extract 128
5.1.4 Results and Discussion 133
5.2 *Michelia champaca* Linn 135
5.2.1 Subcritical CO_2 extraction of flowers 135
5.2.2 Subcritical CO_2 fractions of concrete 135
5.2.2.1 Subcritical CO_2 fraction, first stage 135
5.2.2.2 Subcritical CO_2 fraction, second stage 135
5.2.3 Analysis of the extracts 135
5.1.3.1 GC and GC/MS analysis 135
5.1.3.2 Determination of volatile matter in the extracts 136
5.2.4 Results and Discussion 138
5.3 *Pandanus fascicuaris* Lam 139
5.3.1 Subcritical CO_2 extraction of flowers 139
5.3.2 Subcritical CO_2 fractions of concrete 139
5.3.2.1 Subcritical CO_2 fraction, first stage 140
5.3.2.2 Subcritical CO_2 fraction, second stage 140
5.3.3 Analysis of the extracts 140
5.3.3.1 GC and GC/MS analysis 140
5.3.3.2 Determination of volatile matter in the extracts 141
5.3.4 Results and Discussion 146
5.4 *Murraya paniculata* Linn 147
5.4.1 Subcritical CO_2 extraction of flowers 147
5.4.2 Subcritical CO_2 fractions of concrete 147
5.4.2.1 Subcritical CO_2 fraction, first stage 147
5.4.2.2 Subcritical CO_2 fraction, second stage 147

	5.4.3 Analysis of the extracts	147
	5.1.3.1 GC and GC/MS analysis	147
	5.1.3.2 Determination of volatile matter in the extract	148
	5.4.4 Results and Discussion	151
5.5	*Quisqualis indica* Dent	152
	5.5.1 Subcritical CO_2 extraction of flowers	152
	5.5.2 Subcritical CO_2 fractions of concrete	152
	5.5.2.1 Subcritical CO_2 fraction, first stage	152
	5.5.2.2 Subcritical CO_2 fraction, second stage	152
	5.5.3 Analysis of the extracts	153
	5.5.3.1 GC and GC/MS analysis	153
	5.5.3.2 Determination of volatile matter in the extract	154
	5.5.4 Results and Discussion	157
5.6	Summary	159
CHAPTER-VI: SOLID PHASE MICROEXTRACTION		**161-193**
6.1	Background	161
6.2	Principle of solid phase microextraction	163
	6.2.1 Principle of adsorption on fiber	163
	6.2.2 Desorption of analytes from the SPME fiber	166
6.3	Effect of different fiber coatings	167
	6.3.1 Some variation in use of SPME technique	168
	6.3.2 Fiber coated with PDMS for extraction	169
	6.3.3 Fiber coated with PDMS and porous solid (CAR, DVB or both)	175
6.4	Analysis of floral volatiles	180
	6.4.1 Equilibrium time for adsorption of volatiles emanated from flowers	180
	6.4.2 GC and GC/MS analysis of adsorbed volatiles	180
6.5	*Jasminum sambac* Linn	180
	6.5.1 Headspace analysis of flowers attached to the plant	182
	6.5.2 Picked flower analysis	183
	6.5.3 Results and Discussion	184
6.6	*Michelia champaca* Linn	184
	6.6.1 Headspace analysis of flowers attached to the plant	185

6.6.2 Picked flower analysis 185
6.6.3 Results and Discussion 186
6.7 *Pandanus fascicularis* Lam 187
6.7.1 Picked flower analysis 187
6.7.2 Results and Discussion 188
6.8 *Murraya paniculata* Linn 188
6.8.1 Headspace analysis of flowers attached to the plant 189
6.8.2 Picked flower analysis 189
6.8.3 Results and Discussion 190
6.9 *Quisqualis indica* Dent 191
6.9.1 Headspace analysis of flowers attached to the plant 191
6.9.2 Picked flower analysis 191
6.9.3 Results and discussion 192
6.10 Summary 193
CHAPTER-VII: SUMMARY CONCLUSIONS & FUTURE RESEARCH NEEDS 194-202
7.1 *Jasminum sambac* Linn 194
7.1.1 Essential oil, solvent extracts and subcritical CO_2 extracts 194
7.2 *Michelia champaca* Linn 196
7.2.1 Essential oil, solvent extracts and subcritical CO_2 extracts 196
7.3 *Pandanus fascicularis* Lam 197
7.3.1 Essential oil, solvent extracts and subcritical CO_2 extracts 197
7.4 *Murraya paniculata* Linn 198
7.4.1 Essential oil, solvent extracts and subcritical CO_2 extracts 198
7.5 *Quisqualis indica* Dent 199
7.5.1 Essential oil, solvent extracts and subcritical CO_2 extracts 199
7.6 Energy calculation in different processes 200
7.6.1 Subcritical CO_2 extraction 200
7.6.2 Pentane extraction 201
7.6.3 Hydrodistillation 201
7.7 Conclusions 201
7.8 Future research needs 202
Bibliography 204
Biodata 221