Medicinal compound extraction from the whole body of *Cynodon Dactylon* (L.) Pers. by using green solvents

by

Muhammad Amin Asyraf bin Mohd Amin
15577

Dissertation submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

SEPTEMBER 2015

Universiti Teknologi PETRONAS,
32610, Bandar Seri Iskandar,
Perak Darul Ridzuan.

CERTIFICATION OF APPROVAL

Medicinal compound extraction from the whole body of *Cynodon Dactylon* (L.) Pers. by using green solvents

by

Muhammad Amin Asyraf bin Mohd Amin
15577

A project dissertation submitted to the
Chemical Engineering Programme
Universiti Teknologi PETRONAS
in partial fulfilment of the requirements for the
BACHELOR OF ENGINEERING (Hons)
(CHEMICAL ENGINEERING)

Approved by,

_____

(Dr Pradip Chandra Mandal)

UNIVERSITI TEKNOLOGI PETRONAS
BANDAR SERI ISKANDAR, PERAK
September 2015

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

_____

MUHAMMAD AMIN ASYRAF BIN MOHD AMIN

ABSTRACT

Cynodon Dactylon (L.) Pers. is a common perennial grass which are abundantly found in most subtropical areas. It has been used as traditional remedies to cure wounds, constipation and diarrhoea. Previous researches, especially in India has proved the existence medicinal compounds in some parts of the grass. Therefore, methanolic and ethanolic extraction of the whole body of *C. Dactylon* (L.) Pers. is conducted to produce similar result, hence proving the same occurrence in UTP. In addition to that, ionic liquid extraction from the same parts of the sample by using 1-ethyl-3-methylimidazolium lactate (EMIL) and methanol mixture as the solvent, is performed to study its effectiveness for the process. The extracts are analysed qualitatively Gas Chromatography and Mass Spectrometry (GC-MS). It is found that a higher extraction yield and extracted component variety is resulted from the addition of EMIL to methanol as solvents, and as far as this research is concerned, higher concentration of EMIL further increases the yield as well.

ACKNOWLEDGEMENT

First of all, I am grateful to the Almighty God for lending me a good health during these time taken for me to complete this research.

My sincere appreciation goes to both my parents and siblings for their moral support which has inspired me greatly to finish this project.

I am also thankful and obliged to my Final Year Project (FYP) supervisor, Dr Pradip Chandra Mandal for his earnest and valuable guidance, as well as his willingness to share expertise and advices for me throughout this research period. It has helped me a lot in order for me to conclude this research and to endure my final year in Universiti Teknologi PETRONAS (UTP).

I would also like to take this opportunity to show my thanks to the UTP technicians and staffs that has helped me during this research, especially in laboratory matters. Without them, this project is impossible to be completed.

Last but not least, I want to express my gratitude to my friends, colleagues and any parties that have directly or indirectly given me assistance and support in this venture.

TABLE OF CONTENTS

CERTIFICATION OF APPROVAL ……..…… ii

CERTIFICATION OF ORIGINALITY ……..... iii

ABSTRACT …….. iv

ACKNOWLEDGEMENT ……... v

TABLE OF CONTENTS …… vi

LIST OF ABBREVIATIONS ……. vii

LIST OF FIGURES ……. viii

LIST OF TABLES ……..... vii

CHAPTER 1: INTRODUCTION ……..... 1

1.1 Background Study …….. 1

1.2 Problem Statement ……. 3

1.3 Objectives …….. 4

1.4 Scopes of Study ……..… 4

CHAPTER 2: LITERATURE REVIEW ……..…. 5

2.1 Constituents of *Cynodon Dactylon* (L.) Pers. ……. 5

CHAPTER 3: METHODOLOGY …….……... 7

3.1 Project Flow Chart …….……... 7

3.2 Experimental Procedures …….. 7

CHAPTER 4: RESULT AND DISCUSSION …….……. 10

4.1 Extraction Results …….…….. 11

4.2 Sample Characterization ……..... 13

CHAPTER 5: CONCLUSION AND RECOMMENDATION ……... 17

5.1 Conclusion ……..…… 17

5.2 Recommendation ……..….. 17

REFERENCES ……..…… 18

APPENDICES ……..……. 22

LIST OF ABBREVIATIONS

EMIL	1-ethyl-3-methylimidazolium lactate
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography – Mass Spectroscopy
ISSG	Invasive Species Specialist Group
IUCN	International Union for Conservation of Nature
LC-MS	Liquid Chromatography – Mass Spectroscopy
UTP	Universiti Teknologi PETRONAS

LIST OF FIGURES

FIGURE 1.1	Image of Cynodon Dactylon (L.) Pers.	1
FIGURE 3.1	Flow chart of this project	7
FIGURE 4.1	(A) illustrates the initial state of the extraction while (B) demonstrates the final state for methanolic extraction	10
FIGURE 4.2	Extraction yield vs type of solvent used	12
FIGURE 4.3	Extraction yield vs concentration of EMIL in methanol	12
FIGURE 4.4	GC-MS chromatogram for (a) ethanol, (b) methanol, (c) 0.004 g/mL EMIL and (d) 0.012 g/mL EMIL extracts	13

LIST OF TABLES

TABLE 2.1	Medicinal values of the most common constituents found in C. Dactylon (L.) Pers. (Ashokkumar et al., 2013)	6
TABLE 4.1	Ethanolic extraction data	11
TABLE 4.2	Methanolic extraction data	11
TABLE 4.3	Ionic liquid and methanol mixture extraction data for different mass of ionic liquid	11
TABLE 4.4	Major phytochemical component detected by GC-MS equipment	14

CHAPTER 1

INTRODUCTION

1.1. Background

1.1.1. Cynodon Dactylon (L.) Pers

Cynodon Dactylon (L.) Pers. is a type of perennial grass that sprouts in almost any range of soil types and moisture condition. According to Duke (1983), this species is presumed to originate from East Africa and at present, it can be discovered all over the planet, especially in moderate and subtropical areas. In Malaysia, Bermuda grass is widely used in golf courses and lawns as turf grass. However, it can also be easily found at public areas such as roadsides (Gobilik, Jerome & David, 2013). It is commonly known by a variation of names depending on the location where the plant grows. Some of the most established ones are Bermuda grass, Bahama grass, devil's grass and couch grass. It has been categorised as an invasive species by ISSG of the IUCN Species Survival Commission.

FIGURE 1.1 Image of *Cynodon Dactylon* (L.) Pers.

Despite the invasive behaviour, *Cynodon Dactylon* (L.) Pers. has been reported to be used as folk medications, especially in Indian culture, for numerous ailments such as cough, cramps, diarrhoea, epilepsy, headache, haemorrhage, hypertension, hysteria, insanity and snakebite (Duke, 1983). It also has been discovered to exhibit certain biological traits like antimicrobial and antiviral properties (Solanki & Nagori, 2012). Ashokkumar, Selvaraj and Muthukrishnan (2013) also described that this species displays antibacterial and wound healing characteristics in their research. These findings have raised interests among fellow scholars to find out the phytochemical and pharmacological features that contributes to this plant's remedial application.

1.1.2. Green solvents

Solvents are one of the commonly substance used in chemical industry. Environmental concerns in a chemical plant are usually raised from the usage of non-green solvents in its process. Hence, the development of green alternatives to the existing ones has been rapid due to the increase in environmental awareness among industrial practitioners. Capello, Fischer and Hungerbühler (2007) defined the main objective of green solvents as to minimize the effect of using solvents in the chemical processing towards the environment. However, the extent of how green a solvent is raise several questions to academicians. This is because there may be contradictions between the constraints attributed to the solvent in term of safety, chemical efficiency, health, environment and etc. (Prat et al., 2013). For example, even though methanol is virtually harmless to the environment, safety concerns may present due to its physical and chemical properties during the handling procedure.

Water is generally the greenest solvent that one can get because it is perfectly safe to handle and does not harm the environment (Prat et al., 2013). However, this does not necessarily indicate that it can result to the highest yield for an extraction process. Hence, some other green alternatives are essential to be studied in order to find the optimum solvent that can be used for certain processes. Ionic liquids are among the substances that can be related to when discussing about this subject, though

it is rather controversial. Earle and Seddon (2000) describes ionic liquids as designer solvents, in which their properties and performance can be altered accordingly to suit different type of reactions. They are often recyclable, do not require special apparatus to handle the reactions, and the process yield can be optimized if the correct ionic liquid is selected for a certain reaction. However, it is not always the case. It is reported that certain ionic liquid may produce toxic or hazardous by-products from the reaction that it is involved with, such as the production of 1-butyl-3-methylimidazolium fluoride hydrate during the purification of 1-butyl-3-methylimidazolium hexafluorophosphate (Swatloski, Holbrey & Rogers, 2003). Therefore, the selection of proper ionic liquid is crucial to avoid harm towards the surrounding.

1.2. Problem Statement

Cynodon Dactylon (L.) Pers. has been verified to demonstrate various kind of biological properties, but it is yet to be known whether this species will produce the same behaviour irrespective to the location it grows, despite the differences in its surrounding environmental condition. It is probable because this species is understood to have a high genetic variety in its accessions, though the correlation with geographic attribution is not that firm (Liu et al., 2007). Therefore, this study has been conducted to identify the phytochemical constituents that are present in the whole body of *Cynodon Dactylon* (L.) Pers. species found in Perak, particularly in UTP.

In addition, the extraction of the medicinal compound from the grass' body are typically conducted using alcohol as the solvent as demonstrated by Jananie, Priya and Vijayalakshmia (2011), Shabi, Gayathri, Venkatalakshmi and Sasikala (2010), Karthikeyan, Devadasu and Babu (2015) and Kaleeswaran, Ilavenil, Ravikumar (2010). Water is also normally used in the place of alcohol in some researches as shown by Soraya et al. (2015) as well as Karthik and Ravikumar (2010). At present, no research has been found to be applying an ionic liquid as the solvent medium in the extraction process. Thus, this study can assist in understanding the effectiveness of using an ionic liquid for this particular purpose.

1.3. Objectives

The main objective of this research is to determine the presence of medicinal chemical components in the whole body of *Cynodon Dactylon* (L.) Pers. species found in UTP. In order to achieve this goal, three types of solvent are selected to be used to extract the desired constituents. Hence, the sub-objectives are set to be as follows:

1. Extraction of chemical constituents from the whole body of *Cynodon Dactylon* (L.) Pers. by using ethanol as solvent.
2. Extraction of chemical constituents from the whole body of *Cynodon Dactylon* (L.) Pers. by using methanol as solvent.
3. Extraction of chemical constituents from the whole body of *Cynodon Dactylon* (L.) Pers. by using combination of methanol and ionic liquid (EMIL) at different concentration as solvent.

1.4. Scope of Study

The scope of this paper is limited to only clarifying the medicinal compounds that can be extracted from the whole body of *Cynodon Dactylon* (L.) Pers. that grows in UTP area, without experimentally ascertaining the subsequent health effect of each compounds found towards living organisms. The type of solvents used for the extraction process are specified to be ethanol, methanol, as well as a mixture of and 1-ethyl-3-methylimidazolium lactate. After the extraction is completed, the identification of the constituents is conducted using GC-MS.

CHAPTER 2

LITERATURE REVIEW

2.1 Constituents of *Cynodon Dactylon* (L.) Pers.

The previous findings on the phytochemical constituents of various parts from *Cynodon Dactylon* (L.) Pers. is summarised in Appendix (c). The researches can be categorised into two distinct factions. The first group only conducted preliminary analysis by using proven experimental procedures in determining the general medical components of the specimen (Soraya et al., 2010; Saad et al., 2014; Khlifi et al., 2012) while the second group did a detailed analysis by using high end laboratory equipment such as GC-MS, LC-MS and FTIR (Jananie et al., 2011; Shabi et al., 2010; Kaleeswaran et al., 2010).

Shabi et al. (2010) and Jananie et al. (2011) managed to extract significant amount of hexadecanoic acid ethyl ester, or a derivative of palmitic acid and linoleic acid ester, a derivative of linoleic acid when they used ethanol as the solvent. The former is reported to exhibit antioxidant behaviour while the latter demonstrated inhibitory effects. On the other hand, Kaleeswaran et al. (2010) also found n-tricosane, which are reported to show antimicrobial characteristics (Samadi et al., 2012), in the ethanol extract of the leave of this species.

The researches that only conducted the preliminary analysis has commonly found alkaloids, flavonoids, steroids, triterpenoids, tannins, phenols, glycosides and etc. from various part of the plant such as rhizomes, leaves, root, stem and also the whole plant (Solanki & Nagori, 2012; Kaup et al., 2011; Krishanti et al., 2010; Khlifi

et al., 2012; Abdullah et al., 2012; Saad et al., 2014; Soraya et al., 2015). They managed to detect the presence of similar substances even though a variety of solvents is used in the extraction process i.e. acetone, methanol, ethanol, water and etc. TABLE 2.1 illustrates the medicinal values that is shown several of the components detected.

TABLE 2.1: Medicinal values of the most common constituents found in *C. Dactylon* (L.) Pers. (Ashokkumar et al., 2013)

Constituents	**Medicinal Value(s)**
Alkaloids	Cough medicine, antiarrhythmic, antihypertensive, antitumor
Flavonoids	Anti-viral, anti-cancer, anti-inflammatory, anti-allergic, antioxidant
Steroids	Inflammation treatment
Triterpenoids	Cancer treatment, antioxidants, antibacterial, analgesic
Tannins	Antioxidant, antimicrobial
Phenols	Anticancer, anaesthetic/analgesic
Glycosides	Analgesic, anti-rheumatic, anticancer, anti-inflammatory

For this project, a detailed analysis is required to achieve its objectives. The extraction methods used is mainly soxhlet extraction method for volatile solvent and maceration method for the low volatility or non-volatile ones as demonstrated by the prior researches. Since this project is using both type of solvents, both methods are applied accordingly to achieve a better result. Most extraction process is conducted at room condition.

CHAPTER 3
METHODOLOGY

3.1 Project Flow Chart

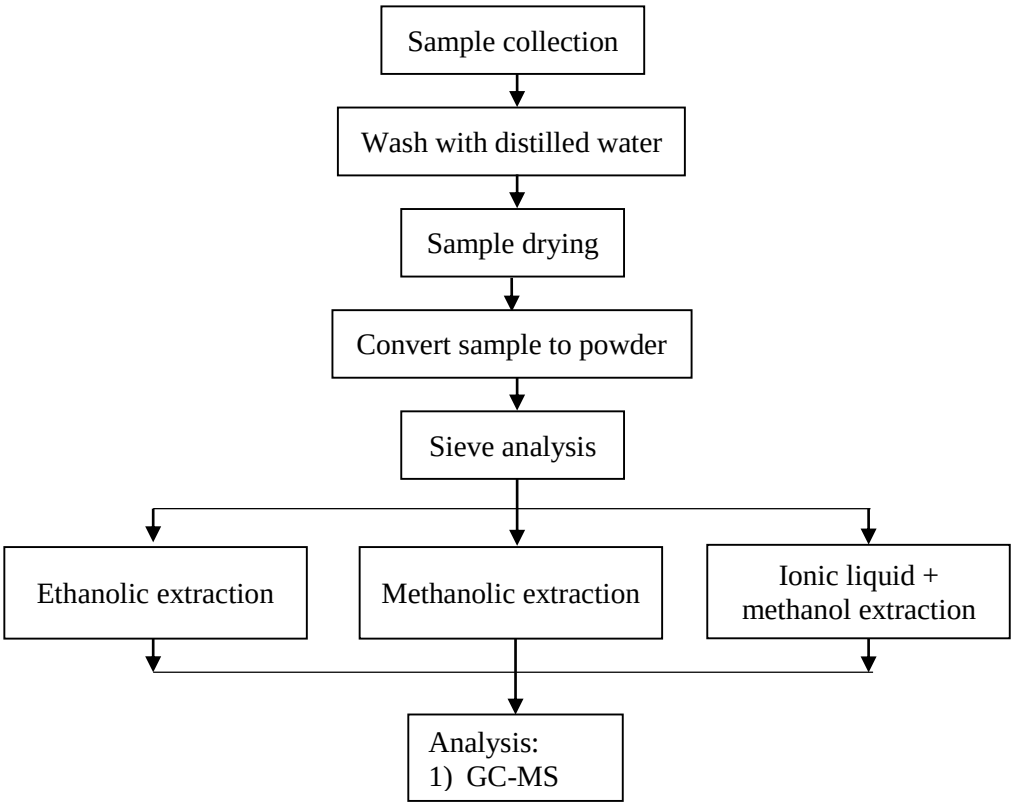


FIGURE 3.1 Flow chart of this project

3.2. Experimental Procedures

This procedure follows the most common techniques applied, in term of the operating condition and the equipment used in the previous literatures based on the similar type of solvent used and the availability of the apparatus and instruments in UTP.

3.2.1. Sample collection

The *Cynodon Dactylon* (L.) Pers. are collected in the area within the compound of UTP, specifically near the motorcycle parking space of Village 5B. The sample species is endorsed by Dr. Pradip Chandra Mandal, the supervisor for this project.

3.2.2. Sample preparation

The collected samples are then washed thoroughly with tap water to remove the soils and any other unwanted solids from the specimens. Then, they are dried in the oven at 60 ^{o}C overnight. Next, the samples are pulverized using an electrical grinder to convert them into powder form to increase the surface area for extraction. Sieve analysis of 250 µm is employed, and only the powder that passes through the mesh will be used for the next stage, so that extraction can take place easily. The powder that does not pass through will be ground again to achieve the desired size.

3.2.3. Preparation of extract

3.2.3.1. Ethanolic extract

5 g of sample is placed in the thimble in a soxhlet apparatus set. Soxhlet extraction is performed by using 250 mL of ethanol for 8 hours under the fume hood at just above the boiling temperature of ethanol (80 ^{o}C). The extract is then stored in the freezer until further use.

3.2.3.2. Methanolic extract

5 g of sample is placed in the thimble in a soxhlet apparatus set. Soxhlet extraction is performed by using 250 mL of methanol for 8 hours under the fume hood at just above the boiling temperature of methanol (65 ^{o}C). The extract is then stored in the freezer until further use.

3.2.3.3. Ionic liquid and methanol mixture extract

5 g of sample is placed in the thimble. The thimble is then placed into a 250 mL conical flask. 1 g of EMIL is dropped into the flask and methanol is filled into the

flask until it reach a volume of 250 mL. A condenser is put on the top of the flask to prevent the mixture from vaporizing to surrounding. Extraction is conducted at just above the boiling temperature of methanol (65 ^{o}C). The extract is then stored in the freezer until further use. The experiment is repeated by increasing the mass of EMIL to 2 g and 3 g respectively.

3.2.4. Analysis of extract

The three extracts (ethanolic, methanolic, methanolic + ionic liquid) of *Cynodon Dactylon* (L.) Pers. is analysed by GC-MS to distinguish the phytochemicals contained in the whole body of the species. Operating condition for the GC-MS are as stated below:

3.2.4.1. GC-MS

An Agilent 5975C GC-MS equipment along with column DB-23 (length 30.0 m, diameter 0.25 mm, film thickness 0.25 μm). The 1μl extract is injected into the GC-MS in split less mode at 200 °C. The column oven temperature is held at 45 °C for 1 minute, then programmed at 5 °C/min until it reaches 280 °C and held for 15 minutes. Helium carrier gas was maintained at a flow rate of 1.4 mL/min. Total GC running time is 48 minutes.

CHAPTER 4

RESULT AND DISCUSSION

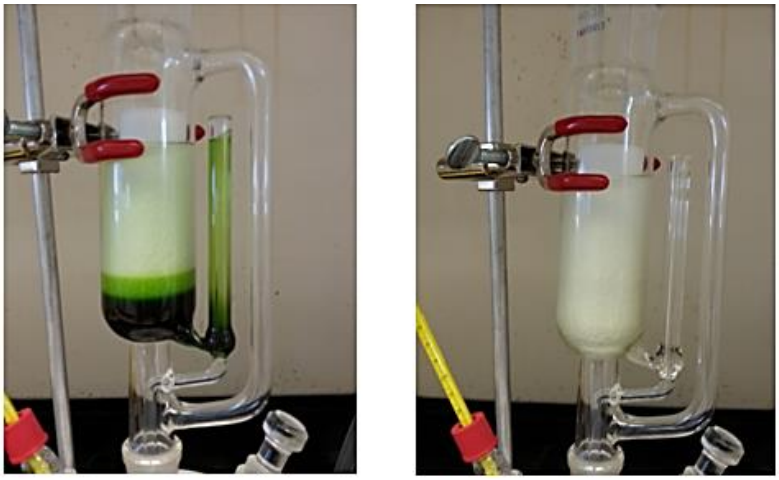


FIGURE 4.1 (A) illustrates the initial state of the extraction while (B) demonstrates the final state for methanolic extraction

The extraction process has been successfully conducted for ethanol, methanol, and a mixture of methanol and of 1-ethyl-3-methylimidazolium lactate (1g, 2g, and 3g) as the solvent. FIGURE 4.1 generally shows that the duration of the procedure of 8 hours is enough to extract the chemical content from the sample. After the completion of extraction for each cases, the amount of chemicals extracted are expressed as the percentage of extraction based on the calculation using the formula as shown below.

$$Percentage\ extraction\ (\%) = \frac{A-B}{A} \times 100\ \% \qquad (1)$$

where A = Mass of thimble + sample before extraction

B = Mass of thimble + sample after extraction

4.1. Extraction Results

4.1.1. Ethanolic extraction at 80 ºC

TABLE 4.1 Ethanolic extraction data

Item	Mass (g)
Sample	5
Empty thimble	3.99
Thimble + sample before extraction	8.99
Thimble + sample after extraction	8.61

Percentage extraction = 4.23 %

4.1.2. Methanolic extraction 65 ºC

TABLE 4.2 Methanolic extraction data

Item	Mass (g)
Sample	5
Empty thimble	3.45
Thimble + sample before extraction	8.45
Thimble + sample after extraction	7.95

Percentage extraction = 5.92 %

4.1.3. Ionic liquid and methanol mixture extraction at 65 ºC

TABLE 4.3 Ionic liquid and methanol mixture extraction data for different mass of ionic liquid

Item	Mass (g)		
	1 g	2 g	3 g
Sample	5.00	5.02	5.03
Empty thimble	3.83	3.47	3.97
Thimble + sample before extraction	8.83	8.49	9.00
Thimble + sample after extraction	8.33	7.62	8.04
Percentage extraction	5.66 %	10.25 %	10.7 %

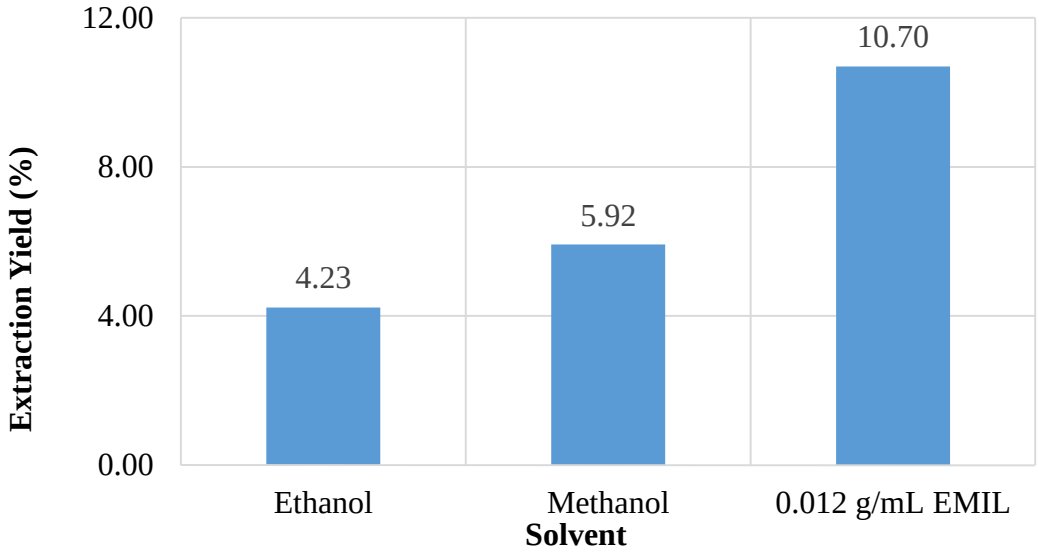


FIGURE 4.2 Extraction yield vs type of solvent used

FIGURE 4.2 demonstrates the percentage of extraction (yield) acquired for each solvent used. It is observed that the extraction of *C. Dactylon* (L.) Pers.by using pure methanol as the solvent obtained a higher yield than using pure ethanol. This is probably due to the lower boiler point of methanol which allows the soxhlet extraction process to experience more cycles throughout the eight-hour period because less energy, hence time, is required for the heating mantle to reach its set temperature before each cycle is restarted.

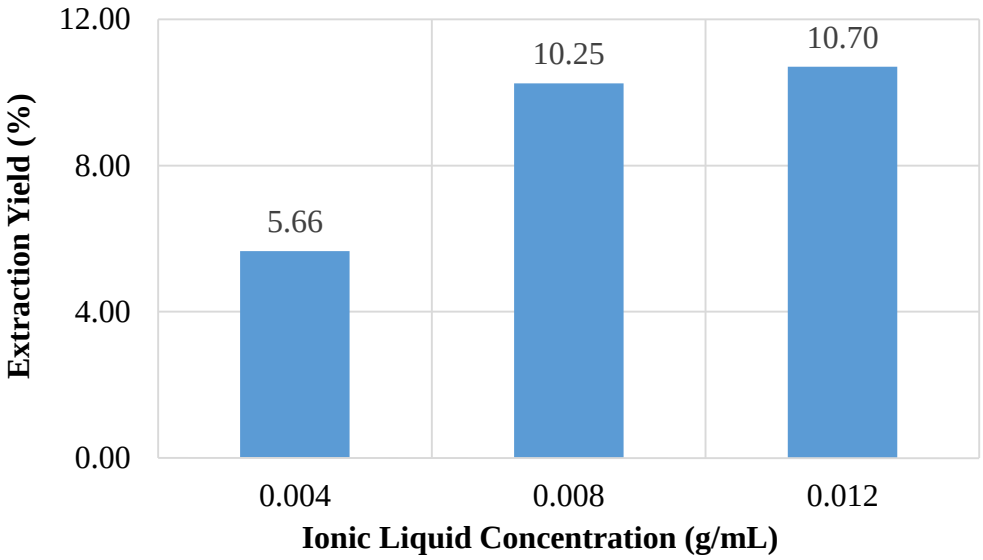


FIGURE 4.3 Extraction yield vs concentration of EMIL in methanol

Moreover, the addition of 1 g of EMIL in methanol (0.004 g/mL EMIL) as the solvent has decreased the percentage of extraction by 0.26 % compared to using pure methanol. However, as the concentration of EMIL is increased to 0.008 g/mL and 0.012 g/mL, the yield improves from 5.92 % to 10.25 % and 10.70 % respectively. The escalation in yield is almost doubled from 0.004 g/mL EMIL to 0.008 g/mL EMIL while there is only a small rise as it is further increased to 0.012 g/mL EMIL. These results perhaps indicate that the optimum EMIL concentration for the Bermuda grass extraction is just above that point. Nonetheless, further experiment is required to confirm this claim.

4.2. Sample Characterization

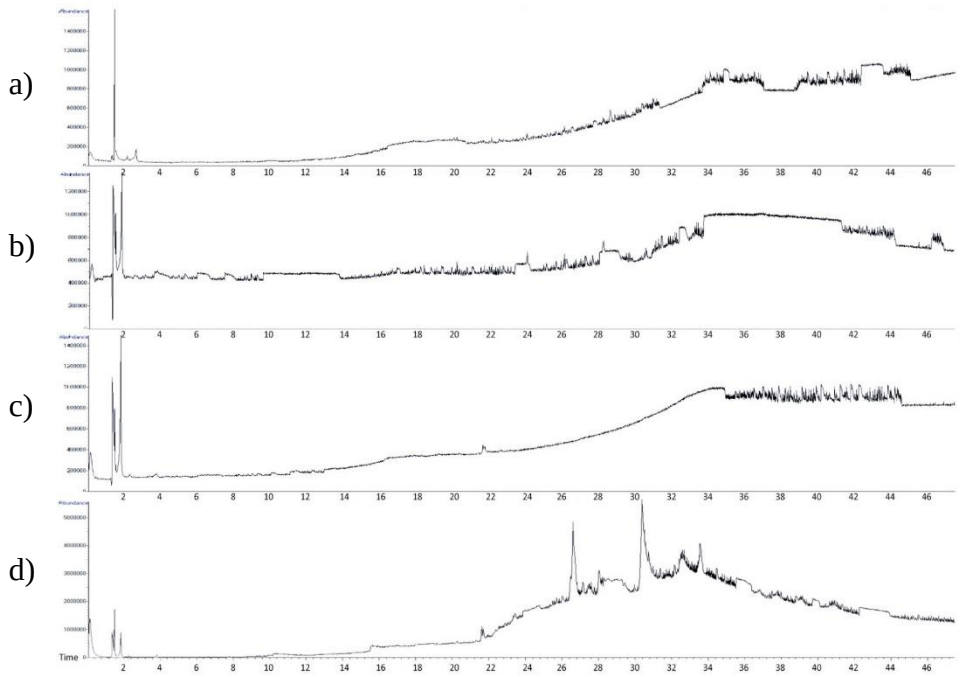


FIGURE 4.4 GC-MS chromatogram for (a) ethanol, (b) methanol, (c) 0.004 g/mL EMIL and (d) 0.012 g/mL EMIL extracts

TABLE 4.4 Major phytochemical component detected by GC-MS equipment

Component Extracted	Peak Area (%)			
	Pure Ethanol	Pure Methanol	EMIL in methanol (g/mL)	
			0.004	0.012
Silane, dimethoxydimethyl-		19.342	16.343	1.518
Silane, diethoxydimethyl-	2.801			
Boric acid, trimethyl ester		16.147	13.750	2.085
Oleic Acid	1.501			1.021
1,2-Benzenedicarboxylic acid, dicyclohexyl ester	1.295			
1,3,12-Nonadecatriene		1.410		
1,9-Tetradecadiene				1.104
2-Methyl-2-docosene			1.142	
Octadec-9-enoic acid	2.121		2.231	
6-Octadecenoic acid, (Z)-	1.579		1.196	
9-Octadecenoic acid, (E)-	1.367			
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester				2.123
Propanenitrile, 3-[2-(4-pyridyl)-1-indolyl)-	1.275			
cis-11-Hexadecenal	1.144			
E-9-Hexadecenal		2.869		
Cyclopropaneoctanal, 2-octyl-	3.399			
Octadecanal, 2-bromo-			2.524	
Methyl 3-hydroxyoctadec-9-enoate			3.091	
Z,Z-6,24-Tritriacontadien-2-one			4.419	
Cyclohexadecane, 1,2-diethyl-			1.213	
9,12-Octadecadien-1-ol, (Z,Z)-				14.455
Z,E-3,13-Octadecadien-1-ol	3.814			
2-Methyl-Z,Z-3,13-octadecadienol				2.335

TABLE 4.4 summarizes the phytochemical components extracted for each solvent types from the chromatograms in Appendix (d), (e), (f) and (g). From the table, it is observed that more components can be identified by the GC-MS equipment when EMIL is added to methanol, compared to using pure methanol. This demonstrates that ionic liquid, in this case, EMIL, can improve separation of chemicals in the extraction process.

The GC-MS was able to detect significant amount of silane, dimethoxydimethyl-, an organosilicon compound, when pure methanol and 0.004

g/mL EMIL is used as solvents respectively, while a small amount of the same substance can be found in 0.012 g/mL EMIL. According to Kregiel and Niedzielska (2014) this substance can greatly improve the antiadhesive and antibacterial properties of a chemically altered polyethylene. Silane, diethoxydimethyl-, which is detected in the pure ethanolic extract can also exhibit the same characteristics, but not as decent as the former one.

Apart from that, boric acid, trimethyl ester, or more commonly known as trimethyl borate, is also extracted in pure methanol as well as in the EMIL solution. This substance is used in synthesizing barbigerone analogues which demonstrates high anti-proliferative behaviour (Wang et al., 2014). Moreover, a significant amount of cyclopropaneoctanal, 2-octyl- is extracted in pure ethanol. This component is also present in methanolic extract of *Moringa Oleifera* leaf (Jayanthi et al., 2015) and *Limonia acidissima* (Pandey, Satpathy & Gupta, 2014), in which these plants shows antioxidant, antibacterial and immunomodulatory potential towards the test subjects. This shows that Bermuda grass may display similar activity on other living organisms.

Furthermore, moderate quantity of oleic acid and its derivative, octadec-9-enoic acid, are able to be extracted in pure ethanol as well as in 0.004 g/mL and 0.012 g/mL EMIL solution. According to Parthasarathy et al. (1990), this constituent may slow progression of atherosclerosis. It also has potential to react positively to skin papillomas (Gustafsson et al., 2004). Substantial amount of 9,12-Octadecadien-1-ol, (Z,Z)- is also found in 0.012 g/mL EMIL solution. This component is a fatty acid derivative and it is alleged to be essential in the diagnosis and regulation of certain diseases such as obesity, cancer and diabetes (Kennedy et al., 2010).

Comparing the constituents found in this study and the one that is obtained previously, it is observed that the specific phytochemical compounds in this research is relatively different to the preceding findings. However, the group that these constituents resided in are fairly similar to the earlier discoveries, such as fatty acids,

oleic acids, and the derivatives of these group, which undoubtedly demonstrates medicinal benefits in certain ways as described.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Cynodon Dactylon (L.) Pers. possess various medicinal benefits despite its invasive behaviour among the plant kingdom. Earlier studies had discovered various phytochemicals and phytoconstituents of Bermuda grass that exhibits medicinal properties, using mostly aqueous and alcoholic extract. This paper aims to verify the contents of this species in UTP compound by investigating the effect of adding ionic liquid to the solvent. It is found that extraction yield can be increased from the addition of EMIL into methanol and more components can be separated from the sample. Significant amount of medicinal compounds such as oleic acid and fatty acid derivatives (9,12-Octadecadien-1-ol, (Z,Z)-) which demonstrates antioxidant, antibacterial and immunomodulatory is successfully extracted from the whole body of *C. Dactylon* (L.) Pers.

5.2. Recommendation

These are some suggestions of the improvements that can be made for future research related to this paper:

1. Conduct the experiment to the extent that the optimum EMIL concentration can be determined.
2. Investigate the effect of other types of ionic liquid towards the extraction process.
3. Administer other analytical techniques to the samples and compare them to increase the accuracy of the result.

REFERENCES

Abdullah, S., Gobilik, J., & Chong, K. P. (2012). Preliminary Phytochemical Study and Antimicrobial Activity from Various Extract of Cynodon Dactylon (L.) Pers. (Bermuda) Against Selected Pathogens. *International Journal of Pharmacy and Pharmaceutical Sciences, 4*(5), 227-230.

Ashokkumar, K., Selvaraj, K., & Muthukrishnan, S. D. (2013). Cynodon dactylon (L.) Pers.: An updated review of its phytochemistry and pharmacology. *J Med Plants Res, 7*, 3477-83.

Bagewadi, Z. K., Siddanagouda, R. S., & Baligar, P. G. (2014). Phytoconstituents investigation by LC-MS and evaluation of antimicrobial and antipyretic properties of Cynodon dactylon. *International Journal of Pharmaceutical Sciences and Research, 5*(7), 2874-2889.

Capello, C., Fischer, U., & Hungerbühler, K. (2007). What is a green solvent? A comprehensive framework for the environmental assessment of solvents. *Green Chemistry, 9*(9), 927-934.

Duke, J. A. (1983). *Cynodon dactylon (L.) Pers*. Handbook of Energy Crops. Unpublished manuscript, Purdue University, West Lafayette, IN.

Earle, M. & Seddon, K. (2009). Ionic liquids. Green solvents for the future. *Pure and Applied Chemistry*, *72*(7), 1391-1398.

Gobilik, J., Jerome, V., & David, D. (2013). Preliminary selection of some ecotypes of Cynodon dactylon (L.) Pers. in Sabah, Malaysia, for turfgrass use. *Journal of Tropical Biology and Conservation, 10*, 51-66.

Gustafsson, L., Leijonhufvud, I., Aronsson, A., Mossberg, A.-K., & Svanborg, C. (2004). Treatment of Skin Papillomas with Topical α-Lactalbumin–Oleic Acid. *New England Journal of Medicine, 350*(26), 2663-2672.

Jananie, R. K., Priya, V., & Vijayalakshmia, K. (2011). Determination of Bioactive Components of Cynodon dactylon by GC-MS Analysis. *New York Science Journal, 4*(4), 16-20.

Jayanthi, M., Garg, S. K., Yadav, P., Bhatia, A. K., & Goel, A. (2015). Some newer marker phytoconstituents in methanolic extract of Moringa oleifera leaves and evaluation of its immunomodulatory and splenocytes proliferation potential in rats. *Indian journal of pharmacology*, *47*(5), 518.

Jegajeevanram, P., Alhaji, N. M. I., & Kumaravel, S. (2014). Identification of Pesticide Compounds of Cynodon Dactylon by GC-MS Analysis. *International Journal of Pharma and Bio Sciences*, *5*(2), 604-608.

Kaleeswaran, B., Ilavenil, S., & Ravikumar, S. (2012). Screening of Phytochemical Properties and Antibacterial Activity of Cynodon dactylon L. *International Journal of Current Research, 3*, 83-88.

Karthik, D., & Ravikumar, S. (2011). Proteome and phytochemical analysis of Cynodon dactylon leaves extract and its biological activity in diabetic rats. *Biomedicine & Preventive Nutrition, 1*(1), 49-56.

Karthikeyan, R., Devadasu, C., & Srinivasa Babu, P. (2015). Isolation, Characterization, and RP-HPLC Estimation of P-Coumaric Acid from Methanolic Extract of Durva Grass (Cynodon dactylon Linn.) (Pers.). *International Journal of Analytical Chemistry, 2015*, 1-7.

Kaup, S. R., Arunkumar, N., Bernhardt, L. K., Vasavi, R. G., Shetty, S. S., Pai, S. R., & Arunkumar, B. (2011). Antihyperlipedemic activity of Cynodon dactylon extract in high-cholesterol diet fed Wistar rats. *Genomic Medicine, Biomarkers, and Health Sciences, 3*(3-4), 98-102.

Kennedy, R. L., Vangaveti, V., Jarrod, G., Shashidhar, V., & Baune, B. T. (2010). Review: Free fatty acid receptors: emerging targets for treatment of diabetes and its complications. *Therapeutic advances in endocrinology and metabolism*, *1*(4), 165-175.

Khlifi, D., Hayouni, E. A., Valentin, A., Cazaux, S., Moukarzel, B., Hamdi, M., & Bouajila, J. (2013). LC–MS analysis, anticancer, antioxidant and antimalarial activities of Cynodon dactylon L. extracts. *Industrial Crops and Products, 45*, 240-247.

Kregiel, D., & Niedzielska, K. (2014). Effect of Plasma Processing and Organosilane Modifications of Polyethylene on Aeromonas hydrophila Biofilm Formation. *BioMed Research International, 2014*, 8.

Krishanti, P. M., Rathinam, X., Kasi, M., Ayyalu, D., Surash, R., Sadasivam, K., & Subramaniam, S. (2010). A comparative study on the antioxidant activity of methanolic leaf extracts of Ficus religiosa L, Chromolaena odorata (L.), King & Rabinson, Cynodon dactylon (L.) Pers. and Tridax procumbens L. *Asian Pacific Journal of Tropical Medicine*, *3*(5), 348-350.

Liu, W., Zhang, X. Q., Li, F., MA, X., & FAN, Y. (2007). Genetic diversity of bermudagrass accessions in south-west China by ISSRs molecular markers and geographic provenance. *Acta Prataculturae Sinica*, *16*(3), 55.

Pandey, S., Satpathy, G., & Gupta, R. K. (2014). Evaluation of nutritional, phytochemical, antioxidant and antibacterial activity of exotic fruit Limonia acidissima. *J Pharm Phytochem*, *3*(2), 81-8.

Parthasarathy, S., Khoo, J. C., Miller, E., Barnett, J., Witztum, J. L., & Steinberg, D. (1990). Low density lipoprotein rich in oleic acid is protected against oxidative modification: implications for dietary prevention of atherosclerosis. *Proceedings of the National Academy of Sciences*, *87*(10), 3894-3898.

Prat, D., Pardigon, O., Flemming, H.-W., Letestu, S., Ducandas, V., Isnard, P., . . . Hosek, P. (2013). Sanofi's Solvent Selection Guide: A Step Toward More Sustainable Processes. *Organic Process Research & Development, 17*(12), 1517-1525.

Saad, R., Appalasamy, L., Khan, J., Kazi, H., Yusuf, E., & Asmani, F. (2014). Phytochemical Screening and Antibacterial Activity of Five Malaysian Medicinal Plants. *British Journal of Pharmaceutical Research, 4*(17), 2019-2032.

Samadi, N., Manayi, A., Vazirian, M., Samadi, M., Zeinalzadeh, Z., Saghari, Z., ... & Khanavi, M. (2012). Chemical composition and antimicrobial activity of the essential oil of Anthemis altissima L. var. altissima. *Natural product research*, *26*(20), 1931-1934.

Shabi, M. M., Gayathri, K., Venkatalakshmi, R., & Sasikala, C. (2010). Chemical Constituents of hydro alcoholic extract and Phenolic fraction of Cynodon dactylon. *International Journal of ChemTech Research, 2*(1), 149-154.

Solanki, R., & Nagori, B. P. (2012). Physiochemical and and Phytochemical Investigation of Whole Plant of Cynodon Dactylon. *Pharmacie Globale (International Journal of Comprehensive Pharmacy), 3*(10), 1-4.

Soraya, H., Moloudizargari, M., Aghajanshakeri, S., Javaherypour, S., Mokarizadeh, A., Hamedeyazdan, S. . . . Garjani, A. (2015). Angiogenic effect of the aqueous extract of Cynodon dactylon on human umbilical vein endothelial cells and granulation tissue in rat. *DARU Journal of Pharmaceutical Sciences, 23*(10), 1-8.

Swatloski, R. P., Holbrey, J. D., & Rogers, R. D. (2003). Ionic liquids are not always green: hydrolysis of 1-butyl-3-methylimidazolium hexafluorophosphate. *Green Chemistry, 5*(4), 361-363.

Wang, G., Wang, F., Cao, D., Liu, Y., Zhang, R., Ye, H., . . . Chen, L. (2014). Synthesis, structure–activity relationships and biological evaluation of barbigerone analogues as anti-proliferative and anti-angiogenesis agents. *Bioorganic & Medicinal Chemistry Letters, 24*(14), 3158-3163.

APPENDICES

a) Project Gantt Chart and Key Milestones for FYP I

Details	Week													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Selection of project topic														
Preliminary research work i. Review on *Cynodon Dactylon* (L.) Pers. species and existing literatures on its constituents ii. Review on previous extraction procedures iii. Review on analytical methods of extract														
Submission of extended proposal						*								
Proposal defence														
Project work continues i. *Cynodon Dactylon* (L.) Pers. sample collection ii. Sample preparation for extraction														
Submission of interim draft report													*	
Submission of interim report														*

Legends

Project work

* Key Milestone

b) Project Gantt Chart and Key Milestones for FYP II

Details	Week														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Project work continues i. Ethanolic extraction ii. Methanolic extraction iii. Ionic liquid + methanol extraction															
Submission of progress report							*								
Project work continues i. Analysis of extract using GC-MS															
Pre-SEDEX										*					
Submission of draft final report											*				
Submission of dissertation (soft bound)												*			
Submission of technical paper												*			
Viva													*		
Submission of project dissertation (Hard Bound)															*

Legends

Project work

* Key Milestone

c) Extraction methods and findings of previous literatures

No	Authors	Parts	Solvent	Extraction and Analysis Information	Findings
1	Karthikeyan R., Devadasu C., Babu P. S. (2015)	Whole plant	Methanol	• Soxhlet extraction + vacuum filtration for 48 hours, 1 kg of powdered parts. • Filtrate centrifuged at 100 ×g for 15 min (4-6 ºC) • RP-HPLC: Mobile phase water → methanol : glacial acetic acid (65 : 34 : 1 v/v), 45 minutes, 1 mL/min, sample size 20 µL	P-coumaric acid
2	Soraya H., Moloudizargari M., Aghajanshakeri S., Javaherypour S., Mokarizadeh A., Hamedeyazdan S., Esmaeli H., Ghaleh G., Mikaili P., Garjani A. (2015)	Rhizomes	Water	• 200 g powder + 2 L distilled water, stirred, for 3 days at 50°C. Filtered three times using the Wattman's paper. Evaporation for 12 hrs at 70°C. Total crude extract: 34 g • Sample size 2-5 mL	Alkaloids, Anthocyanins, Coumarins, Flavonoids, Saponins, Tannins, Phenolic compounds
3	Bagewadi Z. K., Siddanagouda R. S., Baligar P. G. (2014)	Whole plant	Water	• Soxhlet extraction at 60°C for 24 hrs. Extracts concentrated at 45°C with rotary vacuum evaporator. Keep in refrigerator at 4ºC until further use. • HPLC - at 30ºC. Isocratic mode, mobile phase acetonitrile: water (40:60) v/v, flow rate: 1mL min^{-1}; injection volume: 20 µl; UV detection: 274 nm. • FTIR • LC-MS • TLC • H-NMR	Saponins, Tannins, Phenols, Quinones, Glycosides, Carbohydrates, Resins, Coumarins, Proteins & Amino acids
			Methanol		Alkaloids, Flavonoids, Steroids & Triterpenoids, Tannins, Phenols, Quinones, Glycosides, Carbohydrates, Resins, Coumarins, Acidic compounds, Phlabotannins, Catechol
			Petroleum ether		Quinones, Carbohydrates
			Ethanol		Steroids & Triterpenoids, Tannins, Phenols, Quinones, Glycosides, Carbohydrates, Resins, Coumarins, Catechol, Proteins & Amino acids

		Root	Methanol		– cornigerine, $C_8H_{18}O$ i.e. (2-Ethylhexanol), Benzofuran 2-3-dihydro-, $C_9H_{10}O_2$ i.e. (Ethyl benzoate), 2-methyle-4-vinylphenyle, $C_{10}H_9ClO_4$ i.e. Methyl 2-(2-chloroacetoxy) benzoate, Benzaldehyde, 3-(chloroacetoxy) -4-methoxy, Ergosta-7, 22-dien-3β-5α, 6β-triol
4	Jegajeevanram P., Alhaji N. M. I., Kumaravel S. (2014)	Leaves	Ethanol	• 25 g powder soaked in 50 mL ethanol (12 hours) Next, filtered with 2 g sodium sulphate. Then, concentrated through nitrogen flushing. • GC-MS - Sample injected: 2 µl, Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 m x 0.25 mm x 0.25 µm df , Oven temperature: 110° C with 2 min hold, up to 200 °C at the rate of 10 °C/min without hold, up to 280 °C at the rate of 5 °C/min with 9 min hold, Injector temperature 250 °C, Total GC running time 36 min, Inlet line temperature 200°C, Source temperature 200°C Electron energy:70 eV, Mass scan (m/z): 45-450, Solvent Delay: 0-2 min, Total MS running time: 36 min	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- [Synonyms: trans-Squalene] (48.36%), Vitamin E (11.13%), á-Sitosterol (10.49%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (9.40%), 1,2-Benzenedicarboxylic acid, diisooctyl ester (5.93%), Stigmasterol (4.74%)
5	Saad R., Appalasamy L., Khan J., Kazi H., Yusuf E., Asmani F. (2014)	Leaves	Ethanol	• Soxhlet extraction for 24 hours, then dried using rotary evaporator until semi-solid is acquired.	Alkaloids, Steroids and terpenoids, Diterpenes, Tannins and Phenolic compounds, Flavonoids, Anthra-quinone Glycosides
6	Abdullah S., Gobilik J., Chong K. P. (2012)	Whole plant	Acetone	• 100 g soaked into 200 mL of different solvents, shaken on a platform shaker at 150 rpm at 25 °C, for three times. Then evaporated and concentrated under reduced pressure (768	Cardiac glycoside, Tannin
			Chloroform		Cardiac glycoside, Terpenoid and steroid, Saponin, Protein
			Diethyl ether		Alkaloid, Cardiac glycoside

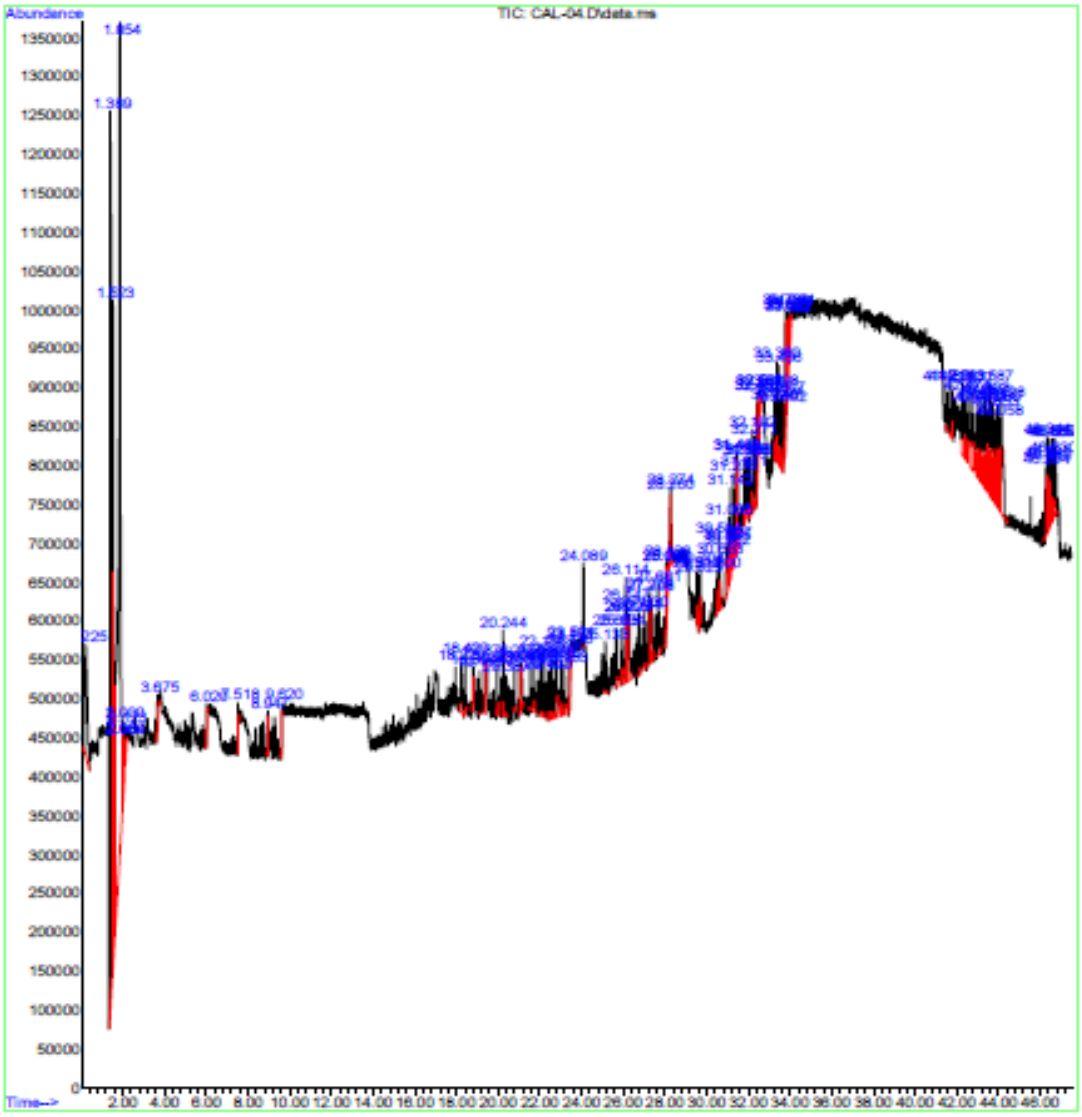
			Ethanol	mmHg – 7 mmHg) to obtain 1 mL of extract per 10 g of plant sample. Aliquot were then kept in -20 ºC temperature for further use.	Cardiac glycoside, Phenolic compound and flavonoid, Tannin, Terpenoid and steroid, Saponin, Carbohydrate (Glucose)
			Ethyl acetate		Cardiac glycoside, Terpenoid and steroid
			n-pentane		Terpenoid and steroid
			Methanol		Cardiac glycoside, Phenolic compound and flavonoid, Saponin, Carbohydrate (Glucose)
7	Khlifi D., Hayouni E. A., Valentin A., Cazaux S., Moukarzel B., Hamdi M., Bouajila J. (2012)	Whole plant	Petroleum ether	• Sample drying in air shade at room temperature, then powdered. Soxhlet extraction of 50 g powder 500 mL of several solvents: petroleum ether (6 h, 40 ◦C), dichloromethane (6 h, 40 ◦C), acetone (6 h, 56 ◦C), methanol/water (3/1) (6 h, 65 ◦C) and water (6 h, 100 ◦C). Next, concentrated by rotary evaporation under vacuum at 35 ◦C	Polyphenols, Tannins, Anthocyanins
			Dichloromethane		Polyphenols, Tannins
			Acetone		Polyphenols, Tannins, Flavonoids, Anthocyanins
			Methanol/water (3/1)		Polyphenols, Tannins, Flavonoids, Anthocyanins
			Water		Polyphenols, Tannins, Flavonoids, Anthocyanins
8	Solanki R., Nagori B. P. (2012)	Root, Stem, Leaf	Benz: CHCl3: MeOH	• 4 g powder soaked in 100 ml solvent for 6 hours, rapidly shaken, and allowed to stand for 18 hours before being rapidly filtered. 25 ml filtrate was taken in a China dish, evaporated to dryness on a water bath at 105° ○ **Water soluble:** distilled water ○ **Alcohol soluble:** ethanol ○ **Hydro alcoholic soluble:** hydro alcoholic solvent system (water : ethanol in a ratio of 60:40)	Steroid (β-sitosterol)
			Pet. Ether: Benz		Steroid (β-sitosterol)
			n-Butanol: Acetic acid: Water		Protein (Amino acids)
			Butanol: Acetone: Water		Alkaloid (Indole alkaloids)
			CHCl3: MeOH: Water		Alkaloid (Ergot alkaloids)
			Ethyl acetate: MeOH: Water		Glycoside
			Toluene: CHCl3: Ethanol		Terpenes (Triterpenoid)
			n-Butanol: Ethanol: Water		Flavonoid (Phenyl-propanoid)
			Glacial acetic acid: Water		Flavonoid (Flavonols and Flavones)

9	Jananie R. K., Priya V., Vijayalakshmia K. (2011)	Leaves	80% alcohol	• 20 g powder soaked in 50 ml of 80% alcohol for 12 hours, then filtered with Whitman filter paper No.41 along with 2g sodium sulphate after being wetted with absolute alcohol. Filtrate was concentrated by bubbling nitrogen gas into the solution to 1 ml. • GC-MS: Ionization energy: 70eV. Carrier gas: Helium (99.999%), 1ml/min, Injection volume: 2µl (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°C. Oven temperature: 110°C (isothermal for 2 min.), with an increase of 10°C /min, to 200°C ,then 5°C/ min. to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra: 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC runtime: 36 minutes	Glycerin (38.49%), 9, 12-Octadecadienoyl chloride, (Z,Z)-(15.61%), Hexadecanoic acid, ethyl ester (9.50%), Ethyl α-d-glucopyranoside (8.42%), Linoleic acid, ethyl ester (5.32%), Phytol (4.89%)
10	Kaup S. R., Arunkumar N., Bernhardt L. K., Vasavi R. G., Shetty S. S., Pa S. R., Arunkumar B. (2011)	Whole plant	80% ethanol	• 500 g powder soaked in ethanol (80% v/v) for 48 at room temperature 28 – 30ºC). Extract was filtered and the filtrate was evaporated to dryness under reduced pressure at 50ºC and stored at 4ºC until use. The extract was dissolved in distilled water to a concentration of 100 mg/ml and stored at -20ºC until use.	Alkaloids, Tannins, Glycoside, Flavonoids, Saponins
11	Kaleeswaran B., Ilavenil S., Ravikumar S. (2010)	Leaves	Ethanol	• Dried leaves were grinded by electrical blender and sieved at 20 µm mesh. Extraction with ethanol by using soxhlet apparatus and with water by cooled maceration. The extraction was carried out for 24 hrs at room temperature with mild shaking. Extract were filtered and concentrated at 45 °C using rotary vacuum evaporator before being vacuum dried. • GC-MS: Column: DB-% ms (length 30.0 m, Diameter 0.25 mm, Film thickness 0.25 µm). The 1µl sample was injected	Tricosane (22.05%), 1,2-Propanediol 3-benzyloxy-1,2-diacetyl (20.30%), Dibutyl phthalate (12.62%), Phthalic acid, Butyl undecyl ester (10.22%), Stigmast-5-En-3-Ol, Oleat (9.13%)

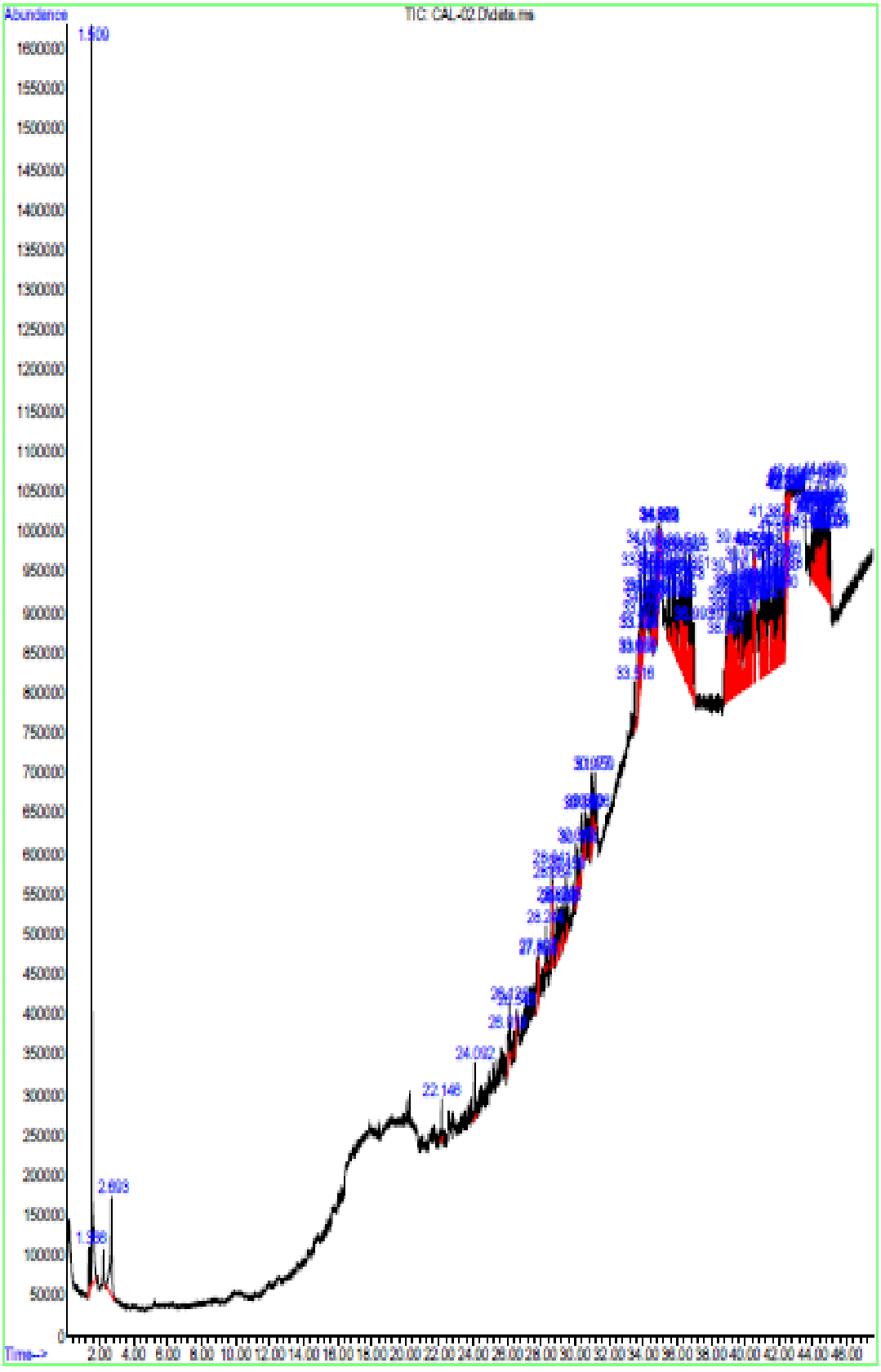
				into the GC-MS (split less mode) at 200 °C. Column oven temperature was held at 45 °C for 1 minute, then programmed at 10 different rates up to 280 °C and held for 15 minutes. Helium carrier gas at 1.4 ml/min.	
12	Karthik D., Ravikumar S. (2010)	Leaves	Water	• 450 g powder was extracted with boiling water for 10 h. Extract was filtered and concentrated in rota vapour under reduced pressure. The concentrated extract was lyophilized to get powder. • GC-MS: Column: DB-% ms (length 30.0 m, diameter 0.25mm, film thickness 0.25_m). The 1µL DG aqueous extract was injected into the GC–MS in split less mode at 200 ◦C. Oven temperature was held at 45 ◦C for 1 min, then at 10 different rates up to 280 ◦C, held for 18 min. Helium carrier gas was maintained at a flow rate of 1.4 ml/min.	Phenylmethanol, 2-propenoic acid (cinnamic acid), sesquiterpene, 2-methoxy-4-prop-2-enylphenyl) acetate, 4',5,7-trihydroxyisoflavone, procyanidin, 3,7,11,15-tetramethyl-2-hexadecen-1-ol
13	Krishanti P M., Rathinam X., Kasi M., Ayyalu D., Surash R., Sadasivam K., Subramaniam S. (2010)	Leaves	Methanol	• Drying at 45ºC for 10 days, then powdered. Then macerated with methanol. Extracts were then filtered by Whitman No 1 paper. Filtrates were concentrated in a rotary evaporator at 40 ºC, then oven dried at for 4 days 40 ºC, then freeze dried for 48 hours, then stored at -20 ºC until use.	Flavonoids
14	Shabi M. M., Gayathri K., Venkatalakshmi R., Sasikala C. (2010)	Whole plant	70% ethanol	• Sample powder was soaked in ethanol: water (70:30) for 72 hours and filtered. The filtrate was concentrated in vacuum. Stored in refrigerator. • 125 ml, 1 M HCl added to 5.0 gm dried plant and soaked at 50 ºC for 30 minutes and at room temperature for 2 hours. The extract was filtered. 50 ml of ether was added to the filtrate and was separated and allowed to evaporate. • GC-MS: 10 mg samples dissolved in methanol. Column type - Elite -5 (5 % diphenyl 95 % dimethyl polysiloxane),	Hexadecanoic acid, ethyl ester (17.49%), Linolenic acid, ethyl ester (11.28%), d-Mannose (11.48%)
			Phenolic fraction		Hydroquinone (69.49%), Levoglucosenone (2.72%), Furfural (6.0%)

				Column dimension 30 m X 0.32 mm), carrier gas: Helium at 1 ml/min, column temperature: 50° C – 285 °C at the rate of 10 °C/min and 5 min hold, at 285 °C, injector and detector temperature: 290 °C, injection mode split, volume injected: 0.5 µL of 2 mg/100 ml in methanol. Run time: 30 minutes. Transfer line temperature: 230 °C, Source temperature: 230 °C, scan range 40 –450 amu	

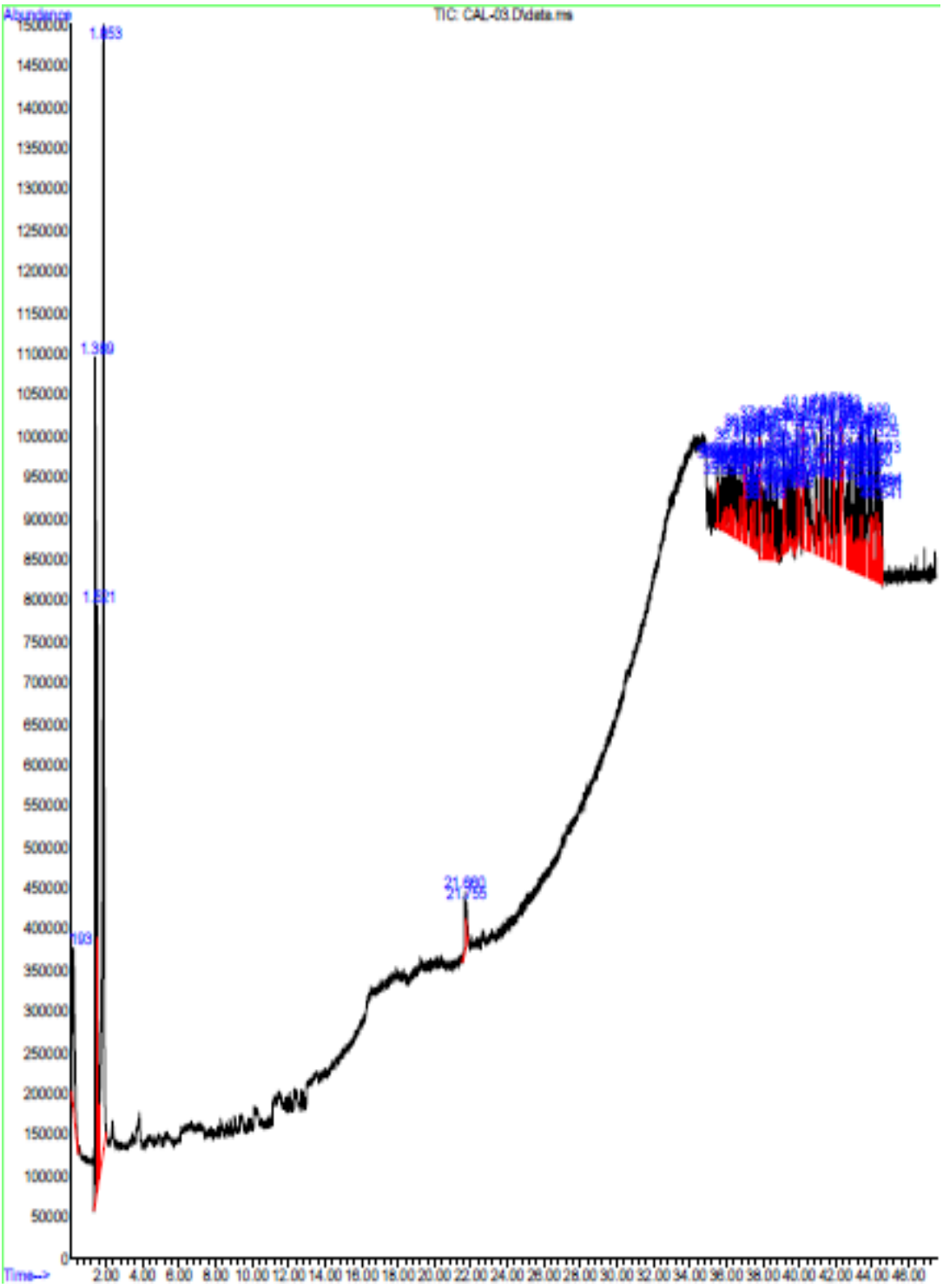
d) GC-MS chromatogram for methanol as solvent



e) GC-MS chromatogram for ethanol as solvent



f) GC-MS chromatogram for 1 g 1-ethyl-3-methyl-imidazolium lactate and methanol mixture as solvent



g) GC-MS chromatogram for 3 g 1-ethyl-3-methyl-imidazolium lactate and methanol mixture as solvents