# الآية



أَنْزَلَ مِنَ وَالْهُوَّمَ الْمُؤْدِمَاءً فَالْحُرْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَالْحُرْرَجْنَا مِنْهُ خَضرِرًا لَهُ حَبَّانُمُ فَتُلْرِ الْحُبُامِورَ مِنَ الْذَخْلِ مِن طَلَاعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنَابٍ لِلْهُ حَبَّانُهُ وَالْدَّ يُتُونَ وَوَلَلْرُمُ مُثَّلَاتَهِ هَا وَ غَيْرانظ وَ اللَّيِّيُ اللَّهُ اللَّ

صدق الله العظيم (99))

### **DEDICATION**

To

My lovely mother

To my father

To my brother and sisters

Acknowledgment

My praise and thanks to Almighty Allah the most Gracious who gave me strength to finish this work.

I would like to express my gratitude to my supervisor professor Mohammed Abdelkarime for this carful supervision ,valuable advices and kindness .

Thanks to my mother for her help and big support.

Thanks are extended to my teachers specially Dr. Adil Alhaj and Dr. Eissa Ismaeel for their help and support.

I would like to express to sincere thanks to my big family especially to my aunts and uncles.

Thanks for my best friend Entesar Khater.

Thanks are also due to all those who helped and encourage me to do this work.

#### **Abstract**

In the present study, the fixed oil of *Pithecellobium dulce* (Roxb.)Benth was extracted from seeds using hot continuous extraction (Soxhlet).

The oil was analyzed by gas chromatography – mass spectrometry (GC-MS)and more than 10 fatty acids were identified the major constituent were : 9,12-octadecadienoic acid (z,z)-,methyl (23.59%) , 9-octadecadienoic acid , methyl ester , (E)-(22,65%) , methyl 20-methylheneicosanoate (13.99%),hexadecanoic acid, methyl ester (12.88%). Minor constituent were tetracosanoic acid, methyl ester (7.45%), methyl stearate (5.78%), methyl 18-methyllnonadecanoate (4.33%), 11-eicosenoic acid , methyl ester (3.57%).

The oil was screened for antibacterial and antifungal activities using six standard human pathogens. Agar well diffusion technique was used to assess the antimicrobial activity of the oil against tow Gram positive (Bacillus subtili and Staphylococcus aureus), two Gram negative (Escherichia coli and Pseudomonas aeroginosa) bacteria and two fungal species (aspergillusniger and Candida albocans).

The result indicate that *Pithecellobium dulce* oil possess bioactive compounds having significant antimicrobial properties.

المستخلص

استخلص الزيت الثابت لنبات التمر الهندي قدرس الزيت بتقنية الكروماتو غرافيا الغازية \_ طيف الكتلة حيث اتضح ان الزيت به المكونات الرئيسية التالية:

9,12-octadecadienoic acid (z,z)-,methyl (23.59%), 9- octadecadienoic acid, methyl ester, (E)-(22,65%), methyl 20-methylheneicosanoate (13.99%),hexadecanoic acid, methyl ester (12.88%).

والمركبات الثانوية هي:

Tetracosanoic acid, methyl ester (7.45%), methyl stearate (5.78%), Methyl 18-methyllnonadecanoate (4.33%), 11-eicosenoic acid, methyl ester (3.57%).

ثم اخضع الزيت لاختيارات بيلوجية \_ كمضاد للميكروبات . وقد استخدمت اربعة انواع من البكتريا القياسية :

Gram positive (Bacillus subtili and Staphylococcus aureus), Gram negative (Escherichia coli and Pseudomonas aeroginosa).

ونوعان من الفطريات:

( aspergillusniger and Candida albocans).

وقد اوضحت نتائج المسح البيولوجي ان الزيت يحتوي علي مواد مثبطة لعمل الميكروبات بصورة واعدة.

الملخص

# Table of Figures

Fig 3.1	Total ion chromatograms
Fig 3.2	Mass spectrum of hexadecanoic methyl ester
Fig 3.3	Mass spectrum of 9,12-octadecanoic acid methyl ester
Fig 3.4	Mass spectrum of 9-octadecanoic acid methyl ester
Fig 3.5	Mass spectrum of methyl stearate

Fig 3.6	Mass spectrum of Cis-11-Eicosenoic acid methyl ester		
Fig 3.7	Mass spectrum of butylated hydroxytoluene		
Fig 3.8	Mass spectrum of 2,2`-Methylene-bis-[6-(1,1-		
	dimethylethyl)-4-methyl]phenol		

## Least of tables

Table 2.1	Test	organisms

- Table 2.1 ..... Oven temperature program
- Table 2.3..... Chromatographic conditions
- Table 3.1..... The typical total ion chromatograms (TIC)

Table 3.2.....Antibacterial activity of *Pithecellobium dulce* oil: M.D.I.Z (mm)

Topic	Page

Table3.3...... Antibacterial activity of standard chemotherapeutic agents: M.D.I.Z (mm).

Table 3.4..... Antifungal activity of standard chemotherapeutic agents against standard fungi.

### Table of contents

الآية	i
Dedication	ii
Acknowledgment	iii
abstract	iv
الملخص	V
Table of figure	vi
Lest of tables	vii
Table of contents	viii- ix
CHAPTER ONE	
Introduction	
1-Introduction	1
1.1- Gas Chromatography	1
1.1.1- Principle of GC	1
1.1.2-GC Advantages and disadvantages	3
1.2-Mass Spectrometry (MS)	5
1.2.1-Principle of mass spectrometry	6
1.2.2-Applications of mass spectrometry	7
1.3- Gas chromatography- Mass spectrometry (GC-MS)	9
1.3.1-Advantages of GC-MS	11
1.3.2-Medical and Pharmaceutical Applications	14
1.3.3-Energy and fuel applications	16
1.4- Essential Oils(EOs)	16
1.4.1- History of essential oils	16
1.4.2-Chemical constituents of essential oils	
1.4.3-Properties of essential oils	19
1.4.4- Pharmacological properties of essential oils	19

1.4.5- Methods of Extracting Essential Oils	20
1.4.5.1-Steam Distillation	20
1.4.5.2- Hot continuous extraction (Soxhlet)	21
1.4.5.3-Maceration	22
1.4.6 -Uses of essential oils	22
1.5-the <i>pithecellobium dulce</i> plant	24
Aim of study	31
CHAPTER TWO	
Materials and Methods	32
2.Materials and Methods	
2.1Materials	32
2.1.1Plant material	32
2.1.2- Instruments	32
2.1.3-Test organisms	32
2.2- Methods	33
2.2.1- Preparations of reagents for phytochemical screening.	33
2.2.2- Preparation of plant extract for phytochemical screening	
2.2.3- Phytochemical screening	
2.2.4-Extraction of oil from seeds of <i>Pithecellobiumdulce</i>	36
2.2.4.1-Esterification of oil	
2.2.5- GC-MS analysis	36
2.2.6-Antimicrobial assay	37
2.2.6.1-Preparation of bacterial suspensions	
2.2.6.2-Preparation of fungal suspensions	
2.2.6.3-Testing for antibacterial activity	
CHAPTER THREE	

Results and Discussion	40
3. and Discussion	
3.1-The GC-MS analysis of <i>Pithecellobiumdulce</i> fixed oil	40
3.2-Antimicrobial activity	45
References	47