

Dedication

Dedicated To

My Parents

Wife

Sisters and Brothers

Acknowledgments

First, I would like to thank Almighty Allah for helping me in finishing this work.

I would like to express my deepest gratitude to my supervisor, Prof. Mohammed Abdelkarim Mohmmed for his support, inspiring guidance, valuable advice and continuous encouragement throughout this study and thank him for his patience during the writing up. I must acknowledge my family my parents, my wife, and my friends for their continual support. I would like also to thank Bahri University for providing the financial support. I would like also to acknowledge the staff of Chemistry Department, College of Science, International Cooperation and Exchange Office, International Islamic University Malaysia for their valuable assistance and help. I would like to acknowledge all the staff of laboratory for infinite support.

My grateful acknowledgement to everyone who has encouraged, helped, shared, contributed and gave me the strength to achieve my goals in this project, especially professor Hassan Elnager, Dr. Anwar Dafalla and Dr. Khalid Hammed.

I would like to thank Almighty Allah for everything.

Abstract

The roots of Sudanese *Albizia Amara* were collected from the Al-Fulla forest in January, 2013 and Barks of *Acacia mellifera* were collected from Niala at west of the Sudan during October, 2013. *Albizia Amara* roots were extracted with 95% ethanol. Sequential solvent extraction using a number of solvents of varying polarity was used for the preliminary separation of flavonoids. Preliminary phytochemical screening of *Albizia Amara* roots indicated the presence of many phyto - components. All fractions showed positive test for alkaloids, saponins, phenolics, carbohydrate and flavonoids. The analysis of the volatiles of the crude and fractions was performed via Gas Chromatography - Mass Spectrometry (Volatile substances varied according to solvent). The ethyl acetate fraction yielded the highest amount of volatile components. In the cup plate agar diffusion assay, all fractions from *Albizia Amara* roots showed inhibitory activity against the *Streptococcus mutans* and *Lacto bacillus*. The ethyl acetate extract showed significant activity on *Streptococcus mutans*, while the n-butanol extract showed high activity on *Lacto bacillus*. Total antioxidant activity and total phenolic content of fractions were measured by different techniques: Ferric reduction activity potential (FRAP), Total antioxidant capacity (TPC), 2, 2 - diphenyl-1-picrylhydrazyl (DPPH), and Cupric reducing antioxidant capacity (ORAC) methods. The results showed that the ethyl acetate fraction has significant antioxidant activity at a mean of (DPPH), 771.83 $\mu\text{g/g}$; (TPC), 3825.47 mg/g ; (CUPRAC) 1902.686 $\mu\text{g/g}$. (FRAP), 538.09 $\mu\text{g/g}$. The chloroform fraction gave the lowest antioxidant activity at mean of: (DPPH) $\mu\text{g/g}$ 24.28; (TPC) 719.48 $\mu\text{g/g}$, (CUPRAC) 75.79 $\mu\text{g/g}$; (FRAP) 132.35 $\mu\text{g/g}$. The ethyl acetate fraction of *Albizia Amara* and n-butanol fraction of *Acacia mellifera* were fractionated by paper chromatography and thin layer chromatography. These four compounds (three from *Albizia Amara* and one from *Acacia mellifera*) were isolated. The structures of these

isolates were elucidated via a combination of spectral techniques: UV, $^1\text{H-NMR}$ and Mass spectroscopy. Furthermore, the four compounds were evaluated for their biological activity. The antibacterial activity was determined by the Well diffusion method against two human pathogens (*Streptococcus mutans*, *Lacto bacillus*). The anti-oxidant capacity was evaluated via two techniques (DPPH and FRAP) and significant results were obtained.

الخلاصة

تم جمع جذور شجرة العرد من الفولا في شهر يناير لعام 2013 اما عينة الكتر من منطقة نيالا في شهر اكتوبر لعام 2013 في غرب السودان. استخلصت جذور شجرة العرد بالايثانول ثم اجرى مسح فيتوكيميائى اوضحت نتائجه وجود القلويدات ، الفينولات، الفلافونيدات ،الكربوهيدرات والصابونينات.

تمت دراسة المواد المتطايره فى مستخلصات: الكحول،ايثيل اسيتات ، بيوتانول ، الايثر والكلوروفورم واتضح ان مستخلص ايثيل اسيتات يحتوى اكبر عدد من المواد المتطايره . اخضعت جميع المستخلصات للاختبار ضد نوعان من البكتريا واتضح ان لمستخلص ايثيل اسيتات فعاليه عاليه ضد البكتريا. ثم اختبرت المستخلصات كمضادات اكسده ،حيث اعطى مستخلص ايثيل اسيتات مقدره عاليه على منع الاكسده التى اختبرت بعدة تقنيات.اما مستخلص الكلوروفورم فقد اعطى اقل فعاليه للاكسده.

اخضع مستخلص ايثيل اسيتات لعينه العرد ومستخلص بيوتانول لعينة الكتر للفصل الكروماتوغرافى بكروموتوغرافيا الورق حيث تم فصل ثلاثه مركبات من عينه العرد ومركب اخر من نبات الكتر ومن ثم تم تحديد البنية التركيبية لهذه النواتج بعدد من التقنيات المطيافيه(طيف الاشعه فوق البنفسجيه ، طيف الرنين النووى المغنطيسى وطيف الكتله).ثم اختبرت المركبات التى تم التعرف عليها ضد الاكسده والبكتريا و اعطت نتائج جيده جدا.

Table of Contents

Dedication		I
Acknowledgement		II
English Abstract		III
Arabic Abstract		IV
List of Contents		V
List of Tables		VIII
List of Figures		IX
Abbreviations		X
Chapter one: Introduction		
1.1	Introduction	1-4
1.2	Natural product as medicine	4.5
1.2.1	Types of natural products	5
	1.2.1.1 Natural product from microorganism	5
	1.2.1.2 Natural product from marine	5-6
	1.2.1.3 Natural product from animal sources	6
	1.2.1.4 Natural product from plant sources	7
1.3	Albizia. Amara	7-8
1.4	Acacia mellifera	9-10
1.5	Flavonoids	11-13
1.6	Flavonoids structure	13-14
1.7	Properties of Flavonoids	14-16
1.8	Human uses of flavonoids	16-17
1.9	Classification of flavonoids	17
1.10	Flavones	18
1.11	Flavonols	20-21
1.12	Flavonones	21-23
1.13	Isoflavonoids	23-24
1.14	Chalcone	24
1.15	Aurones	25
1.16	Anthocyanins	25-27
1.17	Biosynthesis of flavonoids	27-28
1.18	Flavonone formation	28

1.19	Flavones formation	29	
1.20	Isoflavonol formation	29	
1.21	Flavonol formation	30	
1.22	Antioxidant activities of flavonoids	30-32	
1.23	Antimicrobial and antiviral effect of flavonoids	32-34	
1.24	Anticancer effect of Flavonoids	34	
1.25	Anti-diabetic activity of Flavonoids	35-36	
1.26	Anti-inflammatory activity	36	
1.27	Extraction techniques	37-38	
1.28	Phytochemical screening assay:	38	
1.29	Chromatographic techniques	38	
1.29.1	Thin-layer Chromatography(TLC)	38	
1.29.2	Paper Chromatography (PC)	39	
1.29.3	Column Chromatography :	39	
1.29.4	High performance liquid chromatography HPLC	40	
1.30	Identification of flavonoids	40	
1.31	Ultraviolet-Visible spectroscopy	40-42	
1.32	Fourier-transform infrared spectroscopy(FTIR) :	42-43	
1.33	Proton Nuclear Magnetic Resonance spectroscopy	43-46	
1.34	Carbon-13 Nuclear Magnetic Resonance spectroscopy	46-48	
1.35	Mass spectrometry	48-49	
-	Problem statement	50	
-	Significance of the study	50	
1.6	Objective of this study	50	
-	Scope of this study	51	
Chapter Two : Material and Methods			
2.1.	Materials	52	
	2.1.1	Plant material	52
	2.1.2	Materials for chromatographic study	52-53
	2.1.3	Materials for biological screening	53-54
	2.1.4	Material for antioxidant activity	54
	2.1.5	Equipment	55
	2.1.6	Solvents	55
2.2	Methods	56	
	2.2.1	Soxhlet extraction	56
	2.2.2	Solvent- Solvent Extraction:	56-59

	2.2.3	Phytochemical screening	59-61
	2.2.4	Antioxidant activity, Antibacterial Assay and GC-MS screening	61
	2.2.4.1	Determination of Volatile compounds.	61-62
	2.2.4.2	Antioxidant techniques	62-64
	2.2.4.3	Antibacterial Assay	64
	2.2.5	Paper Chromatography	65
	2.2.6.	Structural elucidation of flavonoids	66
	2.2.6.1	UV-Visible Spectroscopy of Isolated Flavonoids	66-67
	2.2.6.1	Shift Reagents for UV spectra of flavonoids .	68
	2.2.6.3	Nuclear Magnetic Resonance Spectroscopy of (NMR)	68-69
	2.2.6.4	Mass Spectrometry	69
	2.2.7	Antioxidant activity, Antibacterial Assay	69
Chapter Three Results and Discussion			
3.1	Extraction of plant phenolics		70
3.2	Qualitative phytochemical analysis		71
3.3	GC-MS analysis		71-76
3.4	Total antioxidant activity and Total phenolic content of fractions		76-79
3.5	Antibacterial activity of different fractions		79-80
3.6	Identification of compound 1		80-86
3.7	Identification of compound 11		86-91
3.8	Identification of compound 111		91-95
3.9	Identification of compound 1v		95-99
3.10	Antibacterial activity		99- 100
3.11	Anti-oxidant capacity		100-101
-	Conclusion		102
-	Recommendations		103
-	References		-

List of tables

No.	Table Name	Page No
1.1	properties of flavonoids with some reagents	16
1.2	Absorption bands for flavonoids	41
1.3	Proton chemical shift ranges of some classes of flavonoids	43-44
1.4	¹³ C chemical shifts for flavonoids	47
1.5	Carbon-13 chemical shift ranges of some classes of flavonoids	48
3.1	Yield of the different crude extracts of A.Amara root	70
3.2	Phytochemical screening of different fractions	71
3.3	Volatile compounds in ethyl acetate fraction	73-74
3.4	Total antioxidant capacity and total phenolic content of different extracts	77
3.5	Antibacterial activity of different fractions	80
3.6	The UV absorption of some flavonoids ¹	81-82
3.7	Antibacterial activity of test compounds (I-IV)	99
3.8	Antioxidant activity of isolated compound(I-IV)	101

List of Figures

No	Figure name	Page No
1.1	Albizia amara	9
1.2	Acacia mellifera	10
2.1	Soxhlet extractor	56
2.2	petroluim ether fraction	58
2.3	Chloroform fraction	58
2.4	Ethyl acetate fraction	59
2.5	n-Butanol fraction	59
2.6	Inhibition of bacterial growth by different extracts	65
3.1	Weight of different fractions	70
3.2	Volatile components in different fractions	72
3.3	GC chromatograms for petroleum ether fraction	74
3.4	GC chromatograms for chloroform fraction	75
3.5	GC chromatogram for ethyl acetate fraction	75
3.6	GC chromatograms for n-butanol fraction	76
3.7	Antioxidant activity of different extracts using (CUPRAC) assay	77
3.8	Antioxidant activity of different extracts using(FRAP) assay	78
3.9	Antioxidant activity of different extracts using(TPC) assay	78
3.10	Antioxidant activity of different extracts using (DPPH) assay	79
3.11	Total antioxidant activity determined by DPPH, TPC, FRAP and CUPRAC methods	79
3.12	UV spectrum of compound I	82
3.13	Sodium methoxide spectrum of compound I	83
3.14	¹ H NMR spectrum of compound I	84
3.15	Sodium acetate spectrum of compound I	84
3.16	Aluminum chloride spectrum of compound I	85
3.17	Boric acid spectrum of compound I	85
3.18	UV spectrum of compound II	87

3.19	Sodium methoxide spectrum of compound II	87
3.20	¹ HNMR spectrum of compound II	88
3.21	Sodium acetate spectrum of compound II	89
3.22	Aluminium chloride spectrum of compound II	89
3.23	Boric acid spectrum of compound II	89
3.24	Mass spectrum of compound II	90
3.25	UV spectrum of compound III	91
3.26	Sodium methoxide spectrum of compound III	92
3.27	¹ HNMR spectrum of compound III	92
3.28	Sodium acetate spectrum of compound III	93
3.29	Aluminum chloride spectrum of compound III	94
3.30	Boric acid spectrum of compound III	94
3.31	UV spectrum of compound IV	95
3.32	Sodium methoxide spectrum of compound IV	96
3.33	¹ HNMR spectrum of compound IV	97
3.34	Sodium acetate spectrum of compound IV	98
3.35	Aluminum chloride spectrum of compound IV	98
3.36	Boric acid spectrum of compound IV	98
3.37	Antibacterial activity of compounds .	100
3.38	Percentage inhibition of test compounds(DPPH)	101
3.39	Inhibition concentration of compounds using(FRAP) assay	101

List of Scheme

NO	Scheme name	Page No
1.1	Classification of flavonoids	19
2.1	A Sequential solvent extraction	57

Abbreviations

DDPH	2, 2 - diphenyl-1-picrylhydrazyl
FRAP	Ferric reduction activity potential
ROS :	Reactive oxygen species
EGCG:	epigallocatechin gallate
TSP :	Thermal separation probe
NIST :	National Institute of Standards
TPC :	Total phenolic content
CUPRAC :	Cupric reducing antioxidant capacity
MHA :	Mueller-Hinton Agar
TAC:	Total antioxidant capacity.
TE :	Trolox equivalents .
GA:	Gallic acid equivalents.

