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Chemical Composition and Antimicrobial Activities of Fruit Extracts of *Ceratonia siliqua*

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Abstract

The aim of this study was to determine the chemical components and antimicrobial activities of water, thanol and hexane extracts of carob fruit. Gas chromatography-mass spectrometry (GC-MS) analysis of the fruit extracts revealed 21, 20, 28 compounds for water, ethanol and hexane extract respectively. The major compounds were alpha.-D-Glucofuranose 1,2:3,5-bis(benzeneboronate) (50.77%), 1H-Pyrazole, 4,5-dihydro-1,3-diphenyl (18.21%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (13.96%) (water extract); Benzoic acid, 4-amino-, 4-acetoxy-2,2,6,6-tetramethyl-1-piperidinyl ester (23.12%), Benzenamine, N-dodecyl-N-methyl (18.37%), (ethanol extract); 9-Octadecenoic acid (Z)-, methyl ester (26.03), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (21.71%), Methyl stearate (16.82%), Hexadecanoic acid, methyl ester (12%) (Hexane extract). The antimicrobial activity of the fruit extracts with different concentrations (100, 80, 60, 40, 20, 10, 5 and 2.5 mg / ml) and selected antibiotic and antifungal were used against four strains of bacteria (two gram-positive: *Bacillus subtilis*; *Staphylococcus aureus* and two gram-negative: *Escherichia coli*; *Pseudomonas aeruginosa*) and one type of fungus (*Candida albicans*) using disc diffusion method. Hexane and ethanol extracts with concentrations 80,60 mg/ml and 100, 80mg/ml respectively showed an inhibition zone 15,12mm and 15,13mm respectively against *Escherichia coli* which is comparable to that of tetracycline at 40mg/ml with an inhibition zone 13mm. Higher inhibition zone for hexane and ethanol extracts (18 and 17mm respectively) at concentration 100mg/ml was against *Escherichia coli* and *Bacillus subtilis* respectively. Ethanol and hexane extracts with concentration lower than 100mg/ml showed activity against *Candida albicans* with an inhibition zone 12 and 10mm compared to ultra griseo fulavin which has no activity at the mentioned concentrations but has antifungal activity at concentration 100mg/ml with an inhibition zone 16mm.

Keywords: *Ceratonia siliqua L;* Carob Fruit; Chemical Composition; Antimicrobial

Introduction

Throughout the ages, humans have relied on nature for their basic needs for the production of food, shelter, clothing, transportation, fertilizers, flavors and fragrances, and medicines [1]. Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigated yet, and their medical activities could be decisive in the treatment of present or future studies [2]. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world [3]. Traditional medicine that has been adopted by other populations (outside its indigenous culture) is often termed complementary or alternative medicine [4]. In ancient Egypt bishop's weed (Ammi majus) was reported to be used to treat vitiligo a skin condition characterized by a loss of Pigmentation [5,6]. A drug has been produced from this plant to treat psoriasis and other skin disorders [6]. Many potent drugs have been derived from flowering plants including for example *Dioscorea species* (diosgenin) from which all anovulatory contraceptive agents have been derived. Reserpine and other antihypertensive and tranquilizing alkaloids from *Rauwolfia species* and Pilocarpine to treat glaucoma and dry mouth derived from a group of South American trees. In the Citrus family two powerful anti-cancer agents from the Rosy Periwinkle (*Catharanthus roseus*), laxative agents from Cassia sp. and a cardio tonic agent to treat heart failure from digitalis species [7]. Three of the major sources of anti-cancer drugs on the market or completing clinical trials are derived from North American plants used medicinally by native Americans. The papaw (*Asimina spp*), the western yew tree (*Taxus brevifolia*), effective against ovarian cancer and the mayapple (*Podophyllum peltatum*) used to combat leukaemia, lymphoma lung and testicular cancer [8]. The natural products are found to be more effective with least side effects as compared to commercial antibiotics for that reason they are used as alternated remedy for treatment of various infections [9]. *Ceratonia siliqua L* derives from Greek keras, horn and Latin siliqua, common name originates from the Hebrew kharuv, from which the Arabic Carob is derived. The genus Ceratonia belongs to the family *Leguminosae (syn. Fabaceae*) of the order Rosales [10]. The pulp represents 90% of the fruit. It has a high content of sugars and tannins and low contents of protein and fat. Carob powder or syrup is used as an ingredient in cakes and cookies and chocolate substitute, contained high levels of carbohydrates (75.92%), protein (6.34%), low level of fat (1.99%), fiber content 7.30%, was rich source of Fe, Ca, Na, K, P and S as well as vitamins E, D, C, Niacin, B6, folic acid and consisted of 11 phenolic compounds. Carob powder is acclaimed ingredient with a marked nutritional value due to its high dietary fiber and phenol compounds. The soluble fibers exert a preventative role against heart disease and lowering serum cholesterol [11].

Materials and Methods

Materials

Plant Material: Carob pods (*Ceratonia siliqua* L) were randomly harvested from various parts of several trees grown in different locations in the plant garden in Kadogli (South of Kordofan); the samples were collected in the morning in December 2018. The plant was authenticated by a plant taxonomist at Elobeid agricultural researches station to be *Ceratonia siliqua***,** same physiological maturity (dark brown) and of uniform shape and size. The fruits were shade-dried, cleaned and grinded by a mechanical grinder. The grounded samples were stored at room temperature to be ready for further extraction.

Methods

Preparation of Plant Extracts: Hundred grams of the dried fruits powder were macerated exhaustively for three days and five hours at room temperature with 1000 ml of ethanol 99% and with 1000ml of distill water separately and respectively. 250g of dried fruits powder were macerated exhaustively for three days at room temperature with 2500 ml hexane. The extracts were filtered and the solvents were left to evaporate, after evaporation of the solvents, solid products with beige color for water extract, dark orange color for ethanol extract and viscous liquid product with dark green color for hexane extract was obtained.

Preparation of Bacterial Suspensions: One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108- 109 C.F.U/ ml. The suspension was stored in the refrigerator at 4°C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate

dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained [12].

Preparation of Fungus Suspension: The fungus culture was maintained on sabouraud dextrose agar, incubated at 25°C for 4 days. The fungus growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used [12].

Bacterial Microorganisms

- NCTC 8236 (Gram + ve bacteria)-Bacillus subtilis
- Staphylococcus aureus-ATCC 25923(Gram +ve Bacteria)
- Escherichia coli-ATCC 25922(Gram -ve bacteria)
- ATCC 27853 (Gram -ve bacteria) Pseudomonas aeruginosa
- National Collection of Type Culture (NCTC), Colindale, England.
- American Type Culture Collection (ATCC) Rockville, Maryland, USA.

Fungus Microorganism

Candida albicans-ATCC7596

GC-MS Analysis Conditions: The qualitative and quantitative analysis of the samples was carried out by using GC/MS technique model (GC/MS-QP2010-Ultra) from Japans 'Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30 m × 0.25 mm × 0.25 µm). The sample was injected by using split mode, helium helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60°C with rate 10°C /min to 300°C as final temperature degree with 3 minutes hold time , the injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C.The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 27 minutes. Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST).

Results and Discussion

Assessment of Antimicrobial Activities of the Extracts

Assessment of antimicrobial activities of *ceratonia siliqua* extracts with different concentrations (100, 80, 60, 40, 20,10,5 and 2.5 mg/ml) were carried out against four types of bacteria (two gram positive: Bacillus Subtilis and staphylococcus and two gram negative: *Escherichia coli* and *Pseudomonas*) and one type of fungus (*Candida albicans*). Tetracycline and ultra griseo fulavin were used with different concentrations (100, 80, 60 and 40 mg/ml) as positive control for bacteria and fungus respectively. The assessment of antimicrobial activities of the plant extracts and tetracycline and ultra griseo fulavin against bacteria and fungus were shown in Tables 1-4 respectively. Hexane extract showed no antibacterial activity against *Escherichia coli* with concentration 40 mg/ml and below. Ethanol extract exhibited no antibacterial activity against *Escherichia coli* with concentrations (60, 40, 20, 10, 5and 2.5mg/ml) and Pseudomonas aeruginosa with all concentrations. The water extract showed no antibacterial activity against the four tested bacteria with the different concentrations. The fruit extracted by hexane and ethanol exhibited partial activity against the tested fungus (*Candida albicans*) with the different concentrations. On the other hand, water extract showed no antifungal activity against the tested fungus (*Candida albicans*) with the different concentrations. The minimum inhibition concentrations (MIC) of ethanol extract was 40mg/ml against staphylococcus and *Bacillus Subtilis* (with inhibition zones 14mm and 15mm respectively). The MIC of hexane extract was 40mg/ml against *Pseudomonas aeruginosa*, *staphylococcus* and *Bacillus Subtilis* (all have inhibition zones 12mm). The MIC of ethanol and hexane extracts was 40mg/ml against the *Candida albicans* (with inhibition zones 12mm and 10mm respectively).

Plant material	Solvent	Concentration mg/ml	E. c	Ps.a	S.a	B.s
Fruit	Water	100	-	-	-	-
Fruit	Ethanol	100	15	-	15	17
Fruit	Hexane	100	18	15	15	13
Fruit	Water	80	-	-	-	-
Fruit	Ethanol	80	13	-	15	15
Fruit	Hexane	80	15	15	13	13
Fruit	Water	60	-	-	-	-
Fruit	Ethanol	60	-	-	14	15
Fruit	Hexane	60	12	13	12	12
Fruit	Water	40	-	-	-	-
Fruit	Ethanol	40	-	-	14	14
Fruit	Hexane	40	-	12	12	12
Fruit	Water	20	-	-	-	-
Fruit	Ethanol	20	-	-	-	-
Fruit	Hexane	20	-	-	-	-
Fruit	Water	10	-	-	-	-
Fruit	Ethanol	10	-	-	-	-
Fruit	Hexane	10	-	-	-	-
Fruit	Water	5	-	-	-	-
Fruit	Ethanol	5	-	-	-	-
Fruit	Hexane	5	-	-	-	-
Fruit	Water	2.5	-	-	-	-
Fruit	Ethanol	2.5	-	-	-	-
Fruit	Hexane	2.5	-	-	-	-

Table 1: Antibacterial activity of fruit extracts against four types of bacteria (inhibition zone in mm).

Plant material	Solvent	Concentration mg/ml	C. a
Fruit	Water	100	-
Fruit	Ethanol	100	12
Fruit	Hexane	100	13
Fruit	Water	80	-
Fruit	Ethanol	80	12
Fruit	Hexane	80	10
Fruit	Water	60	-
Fruit	Ethanol	60	12
Fruit	Hexane	60	10
Fruit	Water	40	-
Fruit	Ethanol	40	12
Fruit	Hexane	40	10

Table 2: Antifungal activity of fruit extracts against *candida albican* (inhibition zone in mm).

Concentration mg/ml	E. c	Ps. a	S.a	B.s
100	30	40	44	38
80	22	31	38	25
60	20	27	36	25
40	13	25	35	21

Table 3: Antibacterial activity of tetracycline (antibiotic) against four types of bacteria (inhibition zone in mm).

Concentration mg/ml	C.a
100	16
80	-
60	-
40	-

Table 4: Antifungal activity of ultra griseo fulavin (antifungal) against *candida albican* (inhibition zone in mm).
E.c = Escherichia coli, Ps.a = Pseudomonas aeruginosa, S.a = Staphylococcus aureus, B.s = Bacillus subtilis, C.a = Candida albicans, Mic= Minimum inhibition concentration.
18 mm and above = very active, 13-18 mm = active, 9-12 mm = partially active, 9 mm and below = Resistant.

Chemical Compounds of Different Extracts

Analysis of water fruit extract by using GC-MS showed the presence of 21 compounds and listed in Table 5. Ethanol fruit extract was analyzed by using GC-MS leading to the identification of 20 compounds. The compounds were shown in Table 6. Analysis by GC-MS identified 28 compounds in hexane fruit extract which are presented in Table 7. The chemical compounds identified in the crude extracts were listed in tables according to their retention time.

ID#	Name	Ret.Time	Area	Area%
1.	2-Cyclopenten-1-one, 2-hydroxy-	3.309	432971	0.16
2.	2,5-Furandione, 3-methyl-	3.489	495821	0.18
3.	2-Furancarboxaldehyde, 5-methyl-	3.687	236269	0.09
4.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	3.801	148560	0.05
5.	1-Decene	3.856	47902	0.02
6.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.299	888328	0.32
7.	Benzofuran, 2,3-dihydro-	7.401	1676302	0.61
8.	5-Hydroxymethylfurfural	8.312	3951036	1.44
9.	Phenol, 2-methoxy-3-(2-propenyl)-	8.954	319448	0.12
10.	1-Tridecene	9.198	207468	0.08
11.	1H-Pyrazole, 4,5-dihydro-1,3-diphenyl-	14.844	50080321	18.21
12.	Hexadecanoic acid, methyl ester	15.296	4999185	1.82
13.	n-Hexadecanoic acid	15.725	957868	0.35
14.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.947	11663793	4.24
15.	9-Octadecenoic acid (Z)-, methyl ester	17	9267006	3.37
16.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.024	38396109	13.96
17.	Methyl stearate	17.209	3400223	1.24
18.	cis-Vaccenic acid	17.41	4105770	1.49
19.	Octadecanoic acid	17.594	727297	0.26
20.	.alpha.-D-Glucofuranose 1,2:3,5-bis(benzeneboronate)	19.162	1.4E+08	50.77
21.	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	25.459	3359435	1.22

Table 5: Chemical compounds of water extract from the fruit of *ceratonia siliqua*.

ID#	Name	Ret.Time	Area	Area%
1	1-Dodecene	6.509	120460	1.1
2	2-Methoxy-4-vinylphenol	8.437	255914	2.35
3	Phenol, 2-methoxy-3-(2-propenyl)-	8.951	123590	1.13
4	1-Tetradecene	9.2	170754	1.57
5	Eugenol	10.174	386583	3.54
6	1-Heptadecene	11.668	137445	1.26
7	Hexadecanoic acid, methyl ester	15.295	239171	2.19
8	n-Hexadecanoic acid	15.729	463286	4.25
9	Hexadecanoic acid, ethyl ester	15.956	405151	3.71

10	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.944	240436	2.2
11	9-Octadecenoic acid (Z)-, methyl ester	16.989	498869	4.57
12	Methyl stearate	17.209	218784	2.01
13	Oleic Acid	17.416	609998	5.59
14	Linoleic acid ethyl ester	17.548	418123	3.83
15	Ethyl Oleate	17.59	927384	8.5
16	Octadecanoic acid, ethyl ester	17.805	570443	5.23
17	Cyclohexane, (3,3-dimethylpentyl)-	19.728	293559	2.69
18	Isoamyl laurate	19.923	304880	2.79
19	Benzoic acid, 4-amino-, 4-acetoxy-2,2,6,6-tetramethyl-1-piperidinyl ester	25.456	2519869	23.12
20	Benzenamine, N-dodecyl-N-methyl-	25.629	2004029	18.37

Table 6: Chemical compounds of ethanol extract from the fruit of *ceratonia siliqua*.

ID#	Name	Ret.Time	Area	Area%
1	1-Hexanol, 2-ethyl-	4.398	6293962	1.52
2	Hexanoic acid, 2-ethyl-, methyl ester	4.519	3055070	0.74
3	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	10.654	4078371	0.99
4	Phenol, 2,4-bis(1,1-dimethylethyl)-	10.893	3668261	0.89
5	Pentadecane	12.886	755577	0.18
6	Methyl tetradecanoate	13.202	2865796	0.69
7	Tetradecane	13.968	1117824	0.27
8	Pentadecanoic acid, methyl ester	14.275	1170637	0.28
9	Pentadecane, 8-hexyl-	14.997	1025113	0.25
10	Hexadecanoic acid, methyl ester	15.304	49590273	12
11	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	15.565	1746452	0.42
12	Hexadecanoic acid, 15-methyl-, methyl ester	15.917	840585	0.2
13	Heptadecanoic acid, methyl ester	16.275	2628951	0.64
14	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.963	89701290	21.71
15	9-Octadecenoic acid (Z)-, methyl ester	17.014	107578810	26.03
16	Methyl stearate	17.223	69487409	16.82
17	Nonadecanoic acid, methyl ester	18.105	1473577	0.36
18	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	18.57	984967	0.24
19	9,12-Octadecadienoyl chloride, (Z,Z)-	18.608	5973550	1.45
20	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	18.734	6253340	1.51
21	cis-11-Eicosenoic acid, methyl ester	18.765	3830733	0.93
22	Eicosanoic acid, methyl ester	18.968	27024548	6.54
23	Triethylene glycol di(2-ethylhexoate)	20.209	1844540	0.45
24	Docosanoic acid, methyl ester	20.585	8547231	2.07
25	Tricosanoic acid, methyl ester	21.35	1282485	0.31
26	Tetracosanoic acid, methyl ester	22.086	6641910	1.61
27	Squalene	22.81	2556866	0.62
28	Hexacosanoic acid, methyl ester	23.486	1155060	0.28

Table 7: Chemical compounds of hexane extract from the fruit of *ceratonia siliqua*.

Conclusion

The hexane extract revealed a wide antibacterial spectrum against most tested bacterial strain up to the concentration 40mg/ml. Ethanol stand as the second effective solvent. Water extract showed no antimicrobial activities. Hexane and ethanol extracts showed limited effect against *Candida albicans*. These findings indicated

that the antimicrobial activity of plant extracts depend on the type of solvent and the type of tested microorganism. The identification of some chemical compounds such as benzoic acid, 4-amino-, 4-acetoxy-2,2,6,6-tetramethyl-1-piperidinyl ester, Benzenamine, N-dodecyl-N-methyl, (ethanol extract); 9-Octadecenoic acid (Z)-, methyl ester, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, Methyl stearate (16.82%), and Hexadecanoic acid, methyl ester, perhaps enhanced the antimicrobial activity of ethanol and hexane extracts.

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