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Antibacterial Activity of *Arum Cyrenicum* Hurby Corms

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Abstract

Bacterial resistance develops due to the overuse of antibiotics and increases due to the adverse effects of these chemicals. This urges the scientists to exchange these antibiotics with alternatives of natural products. This study was aimed at the evaluation of the antimicrobial efficiency of *A. cyreniacum* Hruby against a number of medically important pathogenic bacteria. *Arum cyrenicum* corms were successively extracted with petroleum ether, chloroform and methanol, and were tested for antimicrobial activities against *Escherichia coli* BTC3, *Salmonella typhi* BTC6, *Pseudomonas aeruginosa* BTC10, and St*aphylococcus aureus* BTC15. A considerable antibacterial efficiency of petroleum ether extract of A. *cyrenaicum* corms was specifically against gram-positive bacteria, *Staphylococcus aureus*. The plant extract has bactericidal activity against *Staphylococcus aureus* at (100 mg/ml) petroleum ether and bacteriostatic at (50 mg/ml) of petroleum Ether. Whereas, the methanolic extract of A. *cyrenaicum* corms showed antibacterial activity against gram-negative bacteria, *Pseudomonas auregenosa*, and showed significant bactericidal effect at 100 mg/ml of methanol extract. The results indicate that petroleum ether and methanolic extracts of A. *cyrenaicum* possess compounds with significant antibacterial properties.

Keywords: *Arum cyrenicum*, Efficiency, Antibacterial.

Introduction

Pathogenic bacteria have increased their resistance to Antibacterial drugs noticeably in the last ten years. Bacterial resistance develops due to the overuse of antibiotics and increases due to the adverse effects of these

chemicals (*1*). This urges the scientists to find another alternative that is more effective against pathogens and safe on human body. Most health problems still to be overcome by traditional medicine especially the use of medicinal plants (*2*). In addition to the study of interactions within plant extracts, many recent innovations involve finding plant compounds that synergize with existing antibiotics, particularly as resistance-modifying agents for use against drug-resistant bacteria (*3-5*).

The family Araceae, commonly known as aroids, encompasses 115 genera and about 3300 species. The family is mainly herbs or climbing shrubs and over 90 % tropical; many family members contain poisonous latex, the poison being destroyed by heat. The genera include Acorus (2 spp), Arum (26 spp), Monstera (50 spp), *Dracuncula*, *Amorphophallus*, and *Cryptocoryne*. *Calamus* or sweet flag rhizome is derived from the perennial herb *Acorus calamus*, which is widely distributed in damp situations in Europe and North America (*6*). The family is known to have been used as medicine in all the world especially in the Indian and Chinese systems of medicine for hundreds of years to cure disease especially the central nervous system abnormalities, diabetes, hypolipidemic, antimicrobial, and anticancer (*7-9*).

Researchers have reported the antimicrobial activity of many species to belong to the Araceae family, Studies on *Typhonium flagelliforme* leaves which belong to the Araceae family, demonstrated that this plant had antibacterial activity against Pseudomonas aeruginosa and *Bacillus subtilis* (*10*). In a study on *Anchomanes difformis* (family Araceae), it was reported to contain antimicrobial activity against bacteria and fungi (*11*).

The antimicrobial activity of petroleum ether extract of *Arum maculatum* was reported against *Staphylococcus epidermidis (12)* [(12)]. *Arum maculatum* agglutinin presents pro-inflammatory activity including neutrophil migration in two ways one which is independent of resident cells and another one dependent on the presence of the cell (*13*). *Arum maculatum* tuber lectin showed insecticidal activity (*14*). The saponin extract is an effective antibacterial agent with a particular effect on *Staphylococcus epidermidis* and *Staphylococcus aureus* infection. Alkaloid extract in this genus is an effective anti-microbial agent (*15, 16*). From a previous study *of A. cyreniacum* Hruby, they found this herbal has antioxidant activity, Free radical scavenging activity of leaves and corms showed moderate antioxidant activity of methanolic extract of A. *cyreniacum* (*17*). The objective of the present study

was aimed to evaluate the antimicrobial activities of A. *cyreniacum* Hruby against samples of pathogenic and nonpathogenic bacteria.

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Materials and Methods

The experiments were conducted in a microbiology laboratory at Biotechnology Center Research in Twaisha, Tripoli –Libya.

Plant material

The plant material of *A. cyreniacum* Hruby was collected from the North-Eastern part of Libya namely the El-Jabal El-Akhdar region. The plant was authenticated at The National Herbarium, Botany Department, Faculty of Science, University of Tripoli-Libya, where a voucher specimen (AC 2008) was kept. The dried and grinded corms of plant materials were hot extracted using a Soxhlet apparatus (3) using petroleum ether, chloroform, methanol, and water respectively.

Media and Bacterial culture:

Muller Hinton agar, Muller Hinton broth, Nutrient agar, and Nutrient broth (oxid) are culture Medias used throughout the study. The following pathogenic bacteria selected from stocks available at microbiology labs in Biotechnology center, Tripoli – Libya (*Escherichia coli* BTC3, *Salmonella typhi* BTC10, *Pseudomonas aeruginosa BTC4*, *Staphylococcus aureus* BTC15).

Agar diffusion method

The screening of extracts on antibacterial effect was carried out by determining the zone of inhibition using paper disc (6 mm in diameter, Whatman No.1) diffusion method (*17-19*). Gram-negative bacterial strains were *Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella typhi*, and gram- positive bacteria including *Staphylococcus aureus*. Bacterial suspension for each strain was first grown on nutrient agar plates at 37^{o}C for 18 to 20 hours, then transferred and adjusted using normal saline 0.85% (W/V) to Macfarland standards (10^{8} CFU/ml). The suspension was inoculated to 90 mm diameter Petri dishes that contain sterile cotton swabs, then diluted the extracts at a concentration of 100mg/ml to show the efficacy of active compounds, then sterilized and filtered with 0.45 µm millipore filters. The sterile discs were impregnated with petroleum ether, chloroform, and

methanolic extract solution (0.05ml from 100mg/ml extract) to evaluate at what concentration the extract can show the best antimicrobial efficacy.

Control standard Streptomycin (10µg/disc) was used and dissolved at the same solvents without plant extracts. The inoculated plates contain the test and standard discs were incubated at 37 ^{o}C for 24 h. Tests were performed in duplicate and the mean of the collected data was used throughout the study.

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC):

The MIC and MBC of *A. cyrenaicum* extracts were determined by Broth microdilution method In vitro antibacterial activity in 96 well microtiter plates. Petroleum ether, chloroform, and methanolic extracts were dissolved in 50% Samples were diluted with Mueller Hinton broth at a concentration of 100 mg/ml, using a 100 µl in each well and performing 1:2 serial dilutions starting from100 to 12.25 mg/ml. A sterility control (media only) and a controlled growth (media + bacteria). Each test and growth control wells were inoculated with 5 µl of bacterial suspension (10^8 CFU/ml or 0.5 McFarland). Experiments were performed in duplicate and the microdilution trays were incubated at 37^0C for 17 hours. MIC values were then defined as the lowest concentration of the petroleum ether, chloroform, and methanolic extracts of corms of *A. cyrenaicum*. Determining the MBC, a100 µl was transferred from each well plate that showed no visible growth to a bacteria-free medium, to evaluate at which concentration the extract has bactericidal effects.

Results

Table 1 summarized agar diffusion results. Petroleum ether extract of *A. cyrenaicum* corms had bactericidal activity against gram-positive bacteria. However, the extract effect against *staphylococcus aureus BTC15* showed an inhibition zone of 9 mm diameter at concentration of 100 mg/ml and no effect at all for the gram-negative strains; *Escherichia coli BTC3, Pseudomonas aeruginosa BTC4* and *Salmonella typhi BTC10.* While methanolic extract of *A. cyrenaicum* corms had an antibacterial effect toward gram-negative, *Pseudomonas aeruginosa* BTC4 showing inhibition zone of 9mm diameter and no effect have seen on the growth of *Escherichia coli* BTC3 and *Salmonella typhi BTC10 spp*. On the other hand, chloroform extract exhibited no antibacterial effect on any of the tested strains bacterial.

The screening of antibacterial effect from the extracts carried out by determining the inhibition zone using paper disc (6 mm diameter. Whatman paper No. 1) Diffusion method (n=2). Each value presented as mean ±SE

Table1: Antibacterial activity of *A. cyrenaicum*:

Code	Bacterial strain	Petroleum ether extract	Chloroform extract	Methanolic extract	Control
BTC3	*Escherchia coli*	-ve	-ve	-ve	-ve
BTC4	*Pseudomonas aeruginosa*	-ve	-ve	9±0.5 mm	-ve
BTC10	*Sallmonella spp*	-ve	-ve	-ve	-ve
BTC15	*Staphylococcus aureus*	9±0.5 mm	-ve	-ve	-ve

From Table 2, the results showed that the presence of turbidly in the wells with all concentrations except at 100% concentration; the growth absent on agar Petri dishes of *Staphylococcus aureus* at this concentration. While at 50% concentration the growth of bacteria decreased.

Table.2: MIC of Petroleum ether extract by microdilution method

Code	Bacterial strain	Optical density at different concentrations				MI
		100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	
BTC15	*Staphylococcus aureus*	Clear	Turbid	Turbid	Turbid	100 mg/ml

Table 3 showed that the methanolic extract of *A. cyrenaicum* at 100mg/ml concentration; inhibit growth of *Pseudomonas aeruginosa BTC4*. Whoever, there is no effect on bacteria at lower concentration

Table.3: MIC of the methanolic extract by microdilution method.

Code	Bacterial strain	Optical density at different concentrations				MI
		100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	
BTC4	*Pseudomonas aeruginosa*	Clear	Turbid	Turbid	Turbid	100 mg/ml

Discussion

The results of the current study showed considerable antibacterial activity. The data from plant extract indicates that petroleum ether extract of *A. cyrenaicum* corms had interesting activity against gram-positive strains of *Staphylococcus aureus* with inhibition zone 9±0.5 mm diameter, while this extract had no effect on gram-negative strains; *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella spp*. However, the data from plant extract shows bactericidal activity against *Staphylococcus aureus* at its maximum concentration (100 mg/ml), also it had bacteriostatic activity against the same bacteria at a concentration of (50 mg/ml). These activities could be attributed to the presence of saturated fatty acids in the petroleum ether extract. Saturated fatty acids had shown similar effects to enhance bactericidal and bacteriostatic activities as indicated in previous studies (*17, 20, 21*). The methanolic extract of *A. cyrenaicum* corms had positive activity against gram-negative strains *Pseudomonas aeruginosa* with inhibition zone 9±0.2 mm diameter. Also, it had no effect on other strains of the selected bacteria.

The antibacterial activity against *Pseudomonas aeruginosa* had a bactericidal effect at the top concentration of 100mg/ml of methanol extract, We propose that the antibacterial activity of methanolic extract might be as a result of its polar compounds, polyphenols and flavonoids are available in the Araceae family plant. These compounds are reported as an effective antimicrobial component (*22, 23*). A previous study showed that the *A. cyrenaicum* corms contain phenolic compounds; caffic acid and p-coumaric acid in methanolic extract (*17*). These findings propose the positive effect attributed to the presence of phenolic compounds in methanolic extract, especially caffeic acid and ρ-coumaric acid, Similar studies confirmed the antibacterial activity of these compounds (*24, 25*). The antibacterial activity is proposed to the other bioactive ingredient such as mannose-binding lectin

(Araceous lectin), that plays important role in supporting the immune defense through binding mannose based structural patterns with microbial surface of most of bacteria, fungi and or parasites in addition to the envelop of some viruses (*26*).

Conclusion

The results indicate that petroleum ether and methanolic extracts of *A. cyrenaicum* possess significant antibacterial properties. Further studies are required to purify the bioactive ingredients responsible for the antibacterial activity.

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كفاءة نبات اللوف القوريني Arum cyrenicum كمضاد بكتيري

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الملخص العربي

تنتج عادة مقاومة البكتريا للمضادات الحيوية بسبب سوء استخدامها او عدم التقيد بتعليمات الطبيب، مما يودي الى أثار عكسية تؤثر في كفاءة استخدام المضادات الحيوية. هذه الظاهرة أثارت اهتمام البحاث والعلماء لإجراء المزيد من الأبحاث التي ستقود إلى إيجاد بدائل ناجعه لها تأثير علاجي محدد، والتي عادة ما تكون مستخلصه من مصادر ذات منشأ نباتي حيث انها تعد الأكثر أماناً في استخداماتها وتأثيراتها على البشر.

هدفت الدراسة الى تقييم كفاءة المستخلص نبات اللوف القوريني (***Arum cyrenicum***) كمضاد حيوي ضد مجموعة من البكتريا الممرضة (***Escherichia coli* BTC3**، ***Pseudomonas aeruginosa* BTC4** ، ***Salmonella typhi* BTC10**، ***Staphylococcus aureus* BTC15**. وقد بينت النتائج الأولية فعالية مستخلص الأثير البترولي لنبات اللوف القوريني المحتوي على مركبات غير قطبية عند تركيز (100 ملجم/مل) كمضاد حيوي يوقف نمو بكتيريا موجبة الجرام (***Staphylococcus aureus* *BTC15***)، بينما بينت النتائج تأثير المستخلص الإثير البترولي لنبات اللوف القوريني كمثبط للنمو لذات البكتيريا عند تركيز (50 ملجم/ مل). وأظهرت النتائج فعالية المستخلص الميثلي لنبات اللوف القوريني المحتوي على مركبات قطبية كمضاد حيوي يوقف نمو البكتيريا سالبة الجرام (***Pseudomonas aeruginosa* BTC4**) عند تركيز (100ملحم/مل). وقد خلصت الدراسة الى أن المستخلص البترولي والاثيري للنبات لديهما تأثيراً فعالاً كمضاد حيوي.

الكلمات المفتاحية: اللوف القوريني (*Arum cyrenicum*)، كفاءة، مضاد حيوي.