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Antibacterial Activity of *Arum Cyrenicum* Hurby Corms

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Abstract

Bacterial resistance develops due to overuse of antibiotics in addition to the adverse effects of these chemicals. This urges the scientists to exchange these antibiotics with alternatives from natural products. The study aimed to evaluate the antimicrobial efficiency of *Arum cyrenicum* Hruby against a number of medically important pathogenic bacteria. *A. cyrenicum* corms extracted with petroleum ether, chloroform and methanol, and tested for antimicrobial activity against *Escherichia coli* BTC3, *Salmonella typhi* BTC10, *Pseudomonas aeruginosa* BTC4, and *Staphylococcus aureus* BTC15. A considerable bactericidal efficiency of petroleum ether extract of *A. cyrenicum* corms were specifically against gram-positive bacteria, *Staphylococcus aureus* BTC15 at (100 mg/ml) petroleum ether and bacteriostatic at (50 mg/ml) of petroleum Ether extract. Whereas, the methanolic extract of *A. cyrenicum* corms had bactericidal effect against gram-negative bacteria, *Pseudomonas aeruginosa* BTC4 at (100mg/ml) of methanolic extract. Herby our results indicate that petroleum ether and methanolic extracts of *A. cyrenicum* possess 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). A detailed description for the plant *A. cerynicum* described in our previous publication (12).

Antimicrobial activity of petroleum ether extract of *A. cerynicum* was reported against *Staphylococcus epidermidis* (13). *A. cerynicum* agglutinin presents pro-inflammatory activity including neutrophil migration by two ways one which is independent on resident cells and another one dependent on the presence of the cell (14). *A. cerynicum* tuber lectin showed insecticidal activity (15). The saponin extract is effective antibacterial agent with particular effect on *Staphylococcus epidermidis* and *Staphylococcus aureus* infection. Plant extracts in this genus are an effective anti-microbial agent (16, 17). From previous study of *A. cerynicum* Hruby, they found this herbal have an antioxidant activity, Free radical scavenging activity of leaves and corms showed moderate antioxidant activity of methanolic extract of *A. cyreniacum* (12). The objective of the present study aimed to evaluate the antimicrobial activities of *A. cyreniacum* Hruby against samples of pathogenic and nonpathogenic bacteria.

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 between latitude and longitude 32°24'11.6"N 21°39'57.9"E. The plant authenticated at The National Herbarium, Botany Department, Faculty of Science, at University of Tripoli-Libya, where a voucher specimen (AC 2008) 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.

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 (12,18,19). 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°C for 18 to 20 hours, then transferred and adjusted using normal saline 0.85% (W/V) to Macfarland standards (10⁸ 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 (10 μg /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 °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 from 100 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°C 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, a 100 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 \pm SE.

Code	Bacterial strain	Petroleum ether extract	Chloroform extract	Methanolic extract	Control
BTC3	<i>Escherchia coli</i>	-ve	-ve	-ve	-ve
BTC4	<i>Pseudomonas aeruginosa</i>	-ve	-ve	9 \pm 0.5 mm	-ve
BTC10	<i>Sallmonella spp</i>	-ve	-ve	-ve	-ve
BTC15	<i>Staphylococcus aureus</i>	9 \pm 0.5 mm	-ve	-ve	-ve



Table 2 described the optical density by means of turbidity in the wells at all concentration except at 100% concentration; the growth is absent on agar petri dishes of *Staphylococcus aureus* BTC15. While at 50% concentration the growth of bacteria as 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	<i>Staphylococcus aureus</i>	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	<i>Pseudomonas aeruginosa</i>	Clear	Turbid	Turbid	Turbid	100 mg/ml

Discussion

The results of the current study showed considerable antibacterial activity. However, results from petroleum ether extract of *A. cyrenaicum* corms had a clear effect against gram-positive strains of *Staphylococcus aureus* BTC15, with inhibition zone 9 ± 0.5 mm diameter. While the same extract had no effect on gram-negative strains; *Escherichia coli* BTC3, *Pseudomonas aeruginosa* BTC4 and *Salmonella typhi* BTC10. However, the results 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 concentration of 50% (50 mg/ml). These effects attributed to the presence of the 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 (12,20,21). The methanolic extract of *A. cyrenaicum* corms had positive activity against gram negative strains *Pseudomonas aeruginosa* BTC4 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* BTC4 had bactericidal effect at maximum concentration 100mg/ml of methanol extract. This finding proposes that the antibacterial activity of methanolic extract might be because of its polar compounds, poly phenols and flavonoids are available in Araceae family plant. These compounds reported as effective antimicrobial components (22,23). Previous study showed that the *A. cyrenaicum* corms contain phenolic compounds; caffeic acid and p-coumaric acid in methanolic extract (12). These findings propose the positive effect attributed to the presence of phenolic compounds in methanolic extract, especially caffeic acid and p-coumaric acid that confirmed in a Similar studies had shown 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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