Inhibition of Fungal Plant Pathogens by Aqueous Extracts of Arum cyreniacum

Ahmed A. Abdulrraziq¹, Sami M. Salih¹, Sultan F. Alnomasy²*, Ziyad M. Aldosari² and Bader S. Alotaibi²

¹Department of Biology, Faculty of Education, Omar Al-Mukhtar University, Al-Beida, Libya.
²Department of Medical Laboratories Sciences, College of Applied Medical Sciences in Al-Quwayiyah, Shaqra University, Riyadh, Saudi Arabia.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Arum cyreniacum is an important member of the family of Araceae because of its bio-activities. Hence this work aimed to establish a link between Arum cyreniacum and its uses as bio-control against plant pathogenic fungi which had never hitherto been established. This work was carried out to evaluate the activity of the aqueous extracts of tubers, leaves, and flowers of Arum cyreniacum against three different types of pathogenic fungi, Fusarium solani, Rhizopus microspores and Aspergillus niger. The antifungal activity of the aqueous extracts of Arum cyreniacum was determined by poisoned food technique. The results showed that Arum cyreniacum had an inhibitory effect in a dose-dependent manner on Fusarium solani, Rhizopus microspores, while Aspergillus niger was resistant to all extracts. However, the great inhibition activity against tested fungi was associated with increasing concentrations of the aqueous extracts of Arum cyreniacum. Data in this work indicated that the use of Arum cyreniacum could be a valid alternative for bio-control of plant pathogenic fungi.

Keywords: Arum cyreniacum; bio-control; plant pathogenic fungi.
1. INTRODUCTION

Fungi cause over 80% of total plant diseases that lead to agricultural losses [1,2]. Thus, synthetic fungicides are used for controlling fungal plant diseases. These substances contain effective chemical compounds [3], but fungi show the ability to the rapid development of resistance against those substances. Fungicides have negative effects on the environment by creating an imbalance in the ecosystem, and it is unsafe for human consumption because of plants' retention of the remnants of synthetic fungicides [4,5]. Studies show that there are over 250,000 plant species that can be used as antimicrobials because of their bioactive chemical compounds [6]. Therefore, it has been directed to use plant extracts to combat fungal pathogens because of its ease of obtaining, and the lack of unwanted side effects, as it is considered friendly [7]. Although Libya is considered one of the most biologically diverse countries, bio-activities of many medicinal plants native unexplored [8]. One of these plants is *Arum cyreniacum* of Araceae family, which is native to Al-Jabal Al-Akhdar region in Libya [Fig. 1] [9]. It is used to treat inflammations of the skin and joints such as psoriasis [10].

The genus of *Arum* has different species based on their geographical and regional location. All species of *Arum* such as *A. maculatum*, *A. palaestinum*, *A. hygrophilum* and *A. discoridis* are used to bio-control types of microbes. For example, a conducted study in Turkey indicated that leaf and fruit extracts of *A. maculatum* have an inhibitory effects on different positive and negative bacteria [11]. For instance, another study that was conducted in Palestine showed that ethanolic extract *A. palaestinum* has inhibitory effect against dermatophytes [12]. Moreover, a study that was conducted in Iran demonstrated the possibility of using *A. maculatum* extract to control a fungus *Botrytis cinerea* that causes tomato stem canker disease [13]. Furthermore, findings from a study that was conducted in Jordan confirmed the effectiveness of alcoholic extract of *A. hygrophilum* against plant pathogenic fungi [14]. The crude aqueous extract of *A. discoridis* leaves had a high inhibitory effect against *Candida albicans* [15]. The extract of *A. cyreniacum* can be relied on to control tomato spot disease caused by *Xanthomonas campestris pv. vesicatoria* [16]. Therefore, the study aimed to evaluate the efficacy of aqueous extracts of *Arum cyreniacum* as bio-control against some plant pathogenic fungi which had never hitherto been established.

2. MATERIALS AND METHODS

*Arum cyreniacum* was collected in Al-Baida City, which located in Al-Jabal Al-Akhdar region, Libya. The parts of *Arum cyreniacum* (tubers, leaves, and flowers) was washed with distilled water. Therefore, tubers, leaves, and flowers were kept on 25°C until they become dry and were grinded by an electric grinder.

2.1 Preparation of Aqueous Extracts

The grinded part of *Arum cyreniacum* (200 g) was added into 1000 mL of sterile distilled water. Then was placed on a shaker for 24 h. After that was centrifuged at 3000 rpm for 10 minutes. Filtration was carried out with a filter paper on a Buckner funnel by using a vacuum pump. In order to change the plant extract to the powder, the obtained liquid was placed in the oven at 45°C for 24 h. After evaporation of water, the remaining powder was collected and used to prepare a stock solution at as concentration of 200 mg/mL. The solutions were kept at 4°C before use them [17,18].

2.1.1 Fungal isolates

Fungal isolates (*Fusarium solani*, *Rhizopus microsporus*, *Aspergillus niger*) were provided by the fungal collection at Department of Plant Protection, Omar Al-Mukhtar University.

2.1.2 Antifungal activity test

Effect of plant extracts on pathogen growth was determined using poisoned food technique. A volume of 5 mL from each of tubers, leaves and flowers extracts with the different concentrations (200, 100 and 50 mg/mL) was dispensed separately into 8.5 cm diameter petri dishes and agitated gently with 45 mL of sterile media PDA. The medium was allowed to be solid and inoculated centrally with 5 mm diameter of mycelia plugs of the tested fungi obtained from 7 days old cultures, using a sterile cork borer. Tested fungi were grow on PDA plates inoculated with sterile water that was served as control. All cultures were incubated at 28°C and fungal colony diameters were measured daily for 7 days [19]. Each experiment were replicates three times. Percentage inhibition as follows:

\[
\text{Percentage inhibition} = \frac{R1 - R2}{R1} \times 100
\]

Where, \( R1 \) = Radial diameter of fungus in control plates (PDA + Water);
\( R2 \) = Radial diameter of fungus in the presence of extracts (PDA + extracts).
2.2 Statistical Analysis

The experiments were designed according to the complete random design (CRD). Statistical analysis was performed using Minitab 17 program and ANOVA variance analysis tables. The averages were compared using Tukey’s test at $P <0.05$.

3. RESULTS

The extracts parts of *Arum cyreniacum* were tested against different plant pathogenic fungi. The findings showed that there are differential effects which depending on the type of extract, the used concentrations and tested fungal species. The aqueous extracts of *Arum cyreniacum* parts (tubers and leaves) at a concentration of 100, 150 mg/mL did not show any inhibitory effect towards the tested fungi (Table 1). While leaves extract with concentration of 150 mg/mL inhibited *Rhizopus microspores* with inhibition rate (27%) (Table 1). Moreover, the highest rates of inhibition were against *Rhizopus microsporus* with a rate of (76.4%), (32.9%), (17%) for flowers, leaves and tubers extracts, respectively. *Fusarium solani* with an inhibition rate of (47%), (10.5%), (5.8%) for the same previous extracts respectively. While *Aspergillus niger* was less sensitive to flower and leaf extract with an inhibition rate (23.5%), (9.4%), respectively (Table 1). Although, the tuber extract had no inhibitory effect against this fungus (Table 1). On another hand, flower extract with concentration of 100, 150 mg/mL showed that a good inhibitory effect against pathogenic fungi, with an inhibition range (12.9-50.5%) (Table 1), whereas 100 mg/mL of this extract had no inhibitory effect against *Aspergillus niger* (Table 1). The findings indicated that a concentration of 200 mg/mL of flower extract has superior effect against tested fungi (Fig. 2).

**Table 1. Antifungal activity of *Arum cyreniacum* aqueous extracts (inhibition %)**

| Extract type | Concentration (mg/mL) | Fusarium solani | Rhizopus microsporus | Aspergillus niger |
|--------------|-----------------------|-----------------|----------------------|------------------|
| Tubers       | 100                   | 0.0 f           | 0.0 f                | 0.0 d            |
|              | 150                   | 0.0 f           | 0.0 f                | 0.0 d            |
|              | 200                   | 5.8±0.2         | 17.0±2.0             | 0.0 d            |
|              | 100                   | 0.0 f           | 0.0 f                | 0.0 d            |
| Leaves       | 150                   | 0.0 f           | 27.0±2.0             | 0.0 d            |
|              | 200                   | 10.5±0.5        | 32.9±1.9             | 9.4±0.6          |
|              | 100                   | 14.1±0.4        | 29.4±0.6             | 0.0 d            |
| Flowers      | 150                   | 20.0±2.0        | 50.5±1.5             | 12.9±1.9         |
|              | 200                   | 47.0±3.0        | 76.4±2.4             | 23.5±1.5         |
| Control      | 0.0 f                 | 0.0 f           | 0.0 f                | 0.0 d            |

*Fig. 1. A photo of *Arum cyreniacu***
Fig. 2. Effect of flower extract against *Fusarium solani*, *Rhizopus microspores* and *Aspergillus niger*. Concentration of 200 mg/mL of flower extract has superior effect against fungi than other extracts

4. DISCUSSION

It is possible to find scarce information about Libyan medicinal plants due to the lack of circulating research on biological activities. Therefore, this study provides information about biological activities of one of the most important of Libyan medicinal plants such as *Arum cyraniacum*. This work was conducted for testing the effects of *Arum cyraniacum* aqueous extracts on different plant pathogenic fungi such as *Fusarium solani*, *Rhizopus microspores* and *Aspergillus niger*. The findings in this study showed that it has a good inhibitory activity against those plant pathogenic fungi. [9,16] show the *Arum cyraniacum* extracts have good inhibitory activity that prevent the growth of many microbes [9,16]. This result was in agreement with many studies that show inhibitory activity of different types of *Arum* genus against plant and dermatological fungi [20,12] However, these result was disagreed with study conducted by other researcher that different extracts of *Arum cyraniacum* parts had no inhibitory effect against types of fungi [21] The results from study showed that flower extracts are more efficient than extracts of leaves and tubers. The most effective effect of *Arum cyraniacum* aqueous extracts was observed at a concentration of 200 mg/mL. The reason for this variation in the inhibitory activity of *Arum cyraniacum* extracts is due to different types of tested species of fungi, concentration and the active dissolved compounds [22] *Aspergillus niger* was the most resistant to all extracts, while *Rhizopus microsporus* was the most sensitive to aqueous extracts. The inhibitory activity of *Arum cyraniacum* may be due to the presence of cyanogenic glycosides [23] or due to a presence of substances such as sterols, alkaloids, calcium oxalate, p-coumaric acid, terpenes, flavonoids, caffeic acid [24,25] that have activities in suppressing of microbial growth.

5. CONCLUSION

This study concludes that *Arum cyraniacum* (tubers, leaves and flowers) possesses inhibitory activity against the pathogenic fungi of the plant, and the most efficacy reached with increasing concentration. The best inhibition activity of aqueous extracts against tested fungi was obtained with 200 mg/mL. *Aspergillus niger* was the most resistant, while *Rhizopus microsporus* was the most sensitivity to aqueous extracts. Therefore, Data in this work indicated that the use of *Arum cyraniacum* could be a valid alternative for bio-control of plant pathogenic fungi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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