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Evaluation of the ability of endemic herbal extracts to bio-control some plant pathogenic bacteria: (Araceae of Libya).

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Potentially control of plant diseases in the future can occur by utilizing endemic plant extracts to provide biotic and abiotic environmental conditions that are beneficial for host development and growth which are adverse to pathogen reproduction and evolution. The present study reports the antibacterial activities of three species of endemic herbs of Araceae. The antibacterial activity was determined by methanol and acetone extraction of aerial and underground parts of three endemic herbaceous species in Libya; *Arisarum vulgari*, *Arum cyreniacum* and *Biarum bovei*. The test was conducted by Screening using the disc diffusion technique, against Two various species of plant-pathogenic bacteria; *Agrobacterium* spp. and *Erwinia* spp. According to this study, the most effective way to manage plant-pathogenic bacteria was tuber extracts, especially *B. bovei* tubers. *A. cyreniacum* tuber extracts also showed good efficacy, while *A. vulgaris* extracts were the least effective. On the other hand, the majority of the examined extracts had the greatest impact on *Erwinia* spp. This is the first report to highlight the potential of all species of endemic herbs of Araceae family of Libya in the bio-control of plant pathogens. Thus, endemic herbs of Araceae can be considered to possess strong germicidal properties against plant disease-causing *Agrobacterium* spp. and *Erwinia* spp.

1. Introduction

The requirement for both quality and quantity of agricultural products has increased due to the growing global population, resulting in a sharp rise in chemical germicides to combat crop diseases. Still, in recent years, consumers have grown increasingly worried about the adverse consequences of chemical Bactericides, fungicides and antibiotics on the environment and human health (Lahlali *et al*., 2022; Miller *et al*., 2022). Libya's low soil fertility and limited arable land are obstacles to its agriculture, the identification of appropriate management strategies for pests, natural resources, and seed development is necessary to increase crop productivity (Park, 2016). A study on roughly 150 farms in eastern Libya revealed excessive pesticide and fertilizer pollution in the crops and soil (El-Barasi *et al*., 2010). Plant-pathogenic bacteria can cause up to 40% of crop losses yearly, posing an increasing danger to the world's food security (Savary *et al*., 2012).

Agrobacterium and *Erwinia* genus are considered among the most important causes of plant bacterial diseases, infecting a variety of vegetable, fruit, and grain crops such as crown gall, leafy gall and fire Blight Disease (Gordon *et al*., 2024; Aktepe and Aysan, 2023). These two genera exhibit strong antibiotic resistance, possibly attributed to codon mutations or genes on the nonconjugative plasmid (Fürst *et al*., 2020; Jimenez Madrid and Ivey, 2023). In contrast, research on biological control employing extract plants is gaining tremendous speed, although there are yet few applications in the field (Bielza *et al*., 2020). Such extracts are a promising strategy for the management of plant bacterial pathogens due to their effectiveness, affordability, and eco-compatibility (Fontana *et al*., 2022). However, it calls for great thought when

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selecting the kind of plant and a more accurate assessment of its efficacy (Raymaekers *et al*., 2020; Collinge *et al*., 2022).

The Araceae family of plants is one of the largest plant families worldwide, due to its wide range of members variety (Mayo *et al*., 1997). Additionally, it has demonstrated efficacy in the fight against fungi and bacteria that infect both humans and animals in the area of biological resistance (Lima *et al*., 2021; Leng *et al*., 2022). Some species of this family are naturally endemic in Al-Jabal Al-Khdar eastern Libya, which is still unexplored in terms of their biological activities, However, a local study in 2020 confirmed that a member of this family has good activity against Gram-positive and Gram-negative bacteria pathogenic to humans (Abdulrraziq and Salih, 2020).

This study aimed to investigate the antibacterial activities of plant pathogens of methanol and acetone extract of aerial and underground parts of endemic herbs of Araceae in Libya.

2. Materials and Methods

The plants were gathered (*Arisarum vulgari*, *Arum cyreniacum* and *Biarum bovei*) from the southern areas (Aslantah and Sidi Al-hamry) in Al-Jabal Al-Akhdar, Eastern Libya, Based on their Folk traditional medical use. The plants were identified, confirmed and authenticated according to (Salih and Abdulrraziq, 2024).

2.1. Extraction method:

Each plant material was extracted with slight modifications according to (Shadid, 2018). Each plant material was successfully extracted with a methanol and acetone solvents Three hundred grams of each powder (leaves and Tubers) was soaked in 1.5l of methanol and acetone separately and put on an orbital shaker for 48h. The mixture was then filtered and centrifuged at 3000 rpm for 10 minutes. The next step was filtering using Whitman No. 1 filter paper and drying in a Rotary evaporator to achieve dry ash (Mummed *et al*., 2018). 2g of ash (leaves and Tubers) was dissolved in 10 ml of distilled water to ash a 200 mg/ml concentration.

2.2. Bacterial isolates:

Agrobacterium spp. and *Erwinia* spp. from the collection of Department of Plant Protection, Omar Al-Mukhtar University.

2.2 Antimicrobial susceptibility test:

The antibacterial activity of the extracts was evaluated using Kirby-Bauer method (Hudzicki, 2009). For this investigation, two different genera of plant pathogenic bacteria were used. To ensure uniform sample distribution, the tested bacteria were placed on Mueller-Hinton agar plates in a back and forth motion. The medium of the inoculated bacteria was examined by placing sterile filter paper discs (6 mm) saturated with the extract on their surface. The plates were incubated at 28 °C for 24 h, with three replicates per plate. The diameters of the inhibited zones were measured with a ruler and subtracted from the diameter of the disc.

Statistical Analysis:

The complete random design (CRD) was followed in the creation of the study experiences. ANOVA variance analysis tables and the Minitab 17 application were used for statistical analysis. Tukey's test was used to compare the averages at $P < 0.05$ (Abdulrraziq *et al*., 2023).

3. Results:

Using the disc diffusion method, the antibacterial activity of Araceae extracts from each species of plant was determined. Tables, Figures (1 and 2) provided the sizes of the inhibitory zones that each type of plant produced against agents of bacterial disease agents.

3.1. Effect herbs of Araceae methanol extracts against plant pathogenic bacteria.

All herbal extracts of Aracea were effective against the studied bacterial species, and methanol extracts from leaves and tubers generally showed good levels of inhibition. Depending on the kind of extract (leaves and tubers), the diameters of inhibition differed across plant species and bacterial species. Araceae tuber extracts were more effective than leaf extracts. The most effective of these extracts were *B. bovei* extracts, which showed an inhibition diameter of 3.5 and 2.9 cm against *Erwinia* spp. and *Agrobacterium* spp., respectively. The same plant's leaf extract had good action against the same prior bacterial species, with diameters of inhibition of 2.6 and 2.2 cm, respectively. The diameters of inhibition (2.4 and 1.8 cm) against bacteria belonging to the same preceding species were reported by the tuber extracts of *A. cyreniacum*. The plant's leaf extracts demonstrated identical inhibitory effectiveness against the two tested bacterial species, with a diameter of 1.5 cm. Furthermore, the tuber extracts from *A. vulgari* showed the lowest efficiency when compared to the remaining Araceae herbs (1.2 and 1.0 cm) for the same prior bacterial species. Leaves extract for this type of herb showed no discernible effectiveness against the studied bacterial species.

Table(1): Effect herbs of Araceae methanol extracts at 200 mg/ml (Tuber and leaves) against plant pathogenic bacteria.

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Extract \ Strain		*Agrobacterium* spp.	*Erwinia* spp.
A. vulgari	Tuber	1.0+0.3c	1.0+0.1d
	Leaves	-	-
A. cyreniacum	Tuber	1.8+0.4b	2.4+0.2bc
	Leaves	1.5+0.2bc	1.5+0.5c
B. bovei	Tuber	2.9+0.5a	3.5+0.2a
	Leaves	2.2+0.3ab	2.6+0.1b

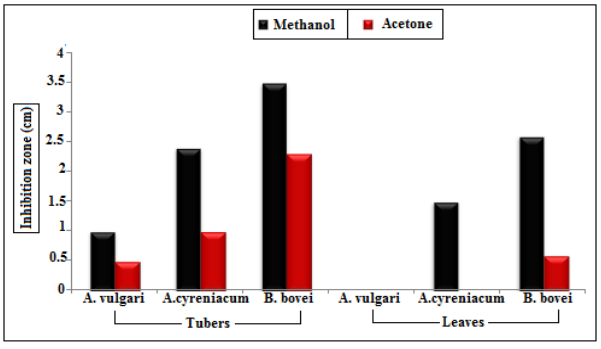
3.2. Effect herbs of Araceae acetone extracts against plant pathogenic bacteria.

The results showed that antibacterial activities were reduced with acetone extracts. Tuber extracts were shown to retain some antibacterial action in most of Araceae plants that were tested. Moreover, *B. bovei* tuber extract had the most inhibitory effect against *Agrobacterium* spp. and *Erwinia* spp., with inhibition diameters of 1.5 and 2.3 cm, respectively. Equal activity was also shown of tuber extracts of *A.cyreniacum* and *A. vulgari* against Agrobacterium with an inhibitory diameter of (1.0 cm). In contrast, extracts from the same herbs that had been tested previously showed only weak efficacy against *Erwinia* spp., with inhibition diameters of (1.0 and 0.5 cm). Conversely, acetone leaf extracts from Araceae herbs could not demonstrate any appreciable efficacy, except for B.boavie leaf extracts against *Erwinia* spp. with a diameter (0.6 cm), and *Agrobacterium* spp. with a diameter (1.0 cm).

Table(2): Effect herbs of Araceae acetone extracts at 200 mg/ml (Tuber and leaves) against plant pathogenic bacteria.

Extract \ Strain		*Agrobacterium* spp.	*Erwinia* spp.
A. vulgari	Tuber	1.0+0.2b	0.5+0.0c
	Leaves	-	-
A. cyreniacum	Tuber	1.0+0.0b	1.0+0.2b
	Leaves	-	-
B. bovei	Tuber	1.5+0.0a	2.3+0.5a
	Leaves	1.0+0.0b	0.6+0.1c

Fig(1): Effect herbs of Araceae methanol and acetone extracts against *Erwinia* spp.



Fig(1): Effect herbs of Araceae methanol and acetone extracts against *Erwinia* spp.

Fig(2): Effect herbs of Araceae methanol and acetone extracts against *Agrobacterium* spp.

4. Discussion:

Araceae family includes three herbaceous species endemic to Libya: *Arisarum vulgare*, *Arum cyrenaicum*, and *Biarum bovei* (Salih and Abdulrraziq, 2024). Traditionally, Libyan folklore uses it as nourishment, as a treatment for psoriasis, and to relieve joint and skin irritation (Ben-ramadan *et al*., 2012; El-Mokasabi, 2014). Nevertheless, through our review of the literature on this family, we could not find any documented scientific reports about the benefits or potential applications. Except for recent studies that dealt with the vital activity of *Arum cyreniacum*. For instance, Abdulrraziq et al. (2021a, 2021b) report that this species is dependable in the fight against plant-pathogenic. This study indicates that indigenous herbs of Araceae have many characteristics that make them effective as germicidal agents for plant disease. The presence of cyanogenic glycosides or other compounds such sterols, alkaloids, calcium oxalate, p-coumaric acid, terpenes, flavonoids, and caffeic acid may be the cause of the inhibitory action herbs of Araceae (Mansour *et al*., 2015; Abdel-Karim *et al*., 2018). Our investigation also showed that tuber extracts were the most efficient means of controlling plant-pathogenic bacteria. The best results were obtained using the methanolic tuber extract of *B. bovei*, however, the acetone extract of the same plant also produced noteworthy results in this investigation. This result was supported by (Wahab *et al*., 2023) who established that the chemical makeup of *B. bovei* validated its traditional use as a major source of nutraceuticals and complementary medicine. Additionally, it has been demonstrated that A phenolic component found in *B.bovei* extract affects the production of chrome in bacteria (Pezeshkpour *et al*., 2016). Additionally, *A. cyreniacum* was efficient against all tested bacterial species, particularly the tuber extracts, this is agreed with the previously described local investigations. As demonstrated by our data, extract *A.vulgari* was the least effective compared to the other extracts; although (Aydin *et al*., 2017) reported high toxicity of this species. In general, both bacterial species were

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impacted by the herbal extracts from Araceae; nevertheless, it seems that *Erwinia* spp. was more susceptible, particularly to methanolic extracts, this is consistent with (Arafat *et al*., 2015) who revealed that some types of extracts plants can decrease fire blight severity on pear trees caused by *Erwinia amylovora*

Conclusion:

Although the health risks associated with chemical substances employed in the bio-control of plant infections are concerning and warrant attention, appropriate alternatives must be investigated to preserve health and lessen their negative effects on the environment. This study focused on the application of Araceae herb extracts in Libya, which can serve as a viable substitute for chemical germicides in biological control management schemes targeting plant diseases.

Conflict of interest: The authors declare that there are no conflicts of interest**.**

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