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Screening of Antibacterial Activity *In vitro* of *Arum maculatum* L. Leaves Extracts

Oussama Mansour^{*1}, Rim Salamma², Lubna Abbas³

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Al Andalus University, Tartous, Syria.

²Department of Pharmacognosy, Faculty of Pharmacy, Tishreen University, Lattakia, Syria.

³Department of Pharmaceutics, Faculty of Pharmacy, Tishreen University, Lattakia, Syria.

*Corresponding author's E-mail: mansouroussama@yahoo.fr

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ABSTRACT

This study was conducted to evaluate the antimicrobial activity of four extracts of *Arum maculatum* L leaves plant belonging to the Aroidae (Araceae) family collected from the coastal surrounding, Lattakia, Syria. Many efforts have been made to discover new antimicrobial compounds from a variety of sources such as micro-organisms, animals and plants. One such source is folk medicine. Systematic screening of them may result in the discovery of novel effective compounds. These extracts were prepared with increasing polarity solvents: petroleum ether, chloroform, ethyl acetate and methanol solvents by steeping extraction method. The antimicrobial activity of the extracts was then assessed using the disk diffusion method against gram-positive (*Staphylococcus aureus*), and gram-negative (*Escherichia coli*) bacteria. The methanolic extracts of *Arum maculatum* L leaves has shown greater antimicrobial activities than the other extracts against tested bacteria, followed by ethyl acetate extracts. The activity was compared with potent broad spectrum antibiotics such as Imepinem.

Keywords: *Arum maculatum*, disk diffusion method, antimicrobial activity, extracts, *Staphylococcus aureus*, *Escherichia coli*.

INTRODUCTION

Anti-microbial agents are undeniably one of the most important therapeutic discoveries of the 20th century. However, mankind is now faced with the global problem of emerging resistance in virtually pathogens.¹ Concern has been expressed about the rising prevalence of pathogenic microorganisms, which are resistant to the newer or modern antibiotics that have been produced in the last three decades.²

The toxic side effects and high cost of new generation antibiotics with limited effective span have resulted in an increase in morbidity and mortality. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity.³

For over thousands of years, natural plants have been considered as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared with the synthetic drug agents. The World Health Organization estimates that 80% of people in developing countries (65% of the world's population) still rely on traditional medicine.^{4,24}

Plants extracts have been rich sources of medicines because they produce a host of bioactive molecules, which probably evolved as chemical defenses against infection.

Most active compounds in these extracts remain unidentified, and their presence is only detected by biological tests.⁵ The identified compounds are products of plant secondary metabolism and belong to the classes of alkaloids, polyphenols, triterpenes, and steroid glycosides.⁶

Arum maculatum L is a common woodland plants species of the Araceae family, which numbers nearly 1000 members, mostly tropical, and many of the mare marsh or water plants.⁷ Being a herbaceous perennial, this is a plant which can grow year after year, the above-ground part of the plant dies away in the winter, but under the ground, part of the plant survives, producing new growth the following spring.⁸

A. maculatum is known by abundance of common names including wild arum, lords and ladies, devils and angels, Adam and Eve, cows and bulls, cuckoo-pint, naked boys, starch-root and wake robin.⁹

It widely grows throughout the coastal surrounding, Syria. The leaves of the plant are used commonly by the local people as vegetable. The roots of the *A. maculatum* species are used in treatment of diaphoretics, expectorants, and vermifuges.¹⁰

Many researchers have been conducted with the aim of studying the biological activities of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant¹¹. This study was aimed at evaluating the antimicrobial property *in vitro* of four extracts of *A. maculatum* L leaves.

MATERIALS AND METHODS

Collection and Identification of plant materials

A. maculatum L. was collected from the coastal Surrounding, Lattakia, Syria during the pre-flowering stage in 2013. The study was carried out at the



Departments of pharmacognosy?? and biology, Faculty of Pharmacy, Tishreen University, Lattakia. Syria.

Plant extracts

The aerial parts of the plant material (leaves) were dried in shade at room temperature and then grounded to a fine powder in a mechanic grinder. The powdered plant materials (30g) were then extracted with 300mL of each solvent (petroleum ether, chloroform, ethyl acetate and 70% methanol) for 72 hours by Steepingmethod.^{12,3} Following filtration with Whatman filter paper(No 1), all extracts were concentrated and evaporated to dryness at room temperature. The yields from the different extracts were weighed and dissolved in dimethyl sulphoxide (DMSO) to form a mother solution (500 mg/1ml) for each extract, then three dilutions (20,50,100 mg/ml) were made from each mother solution. All extracts were maintained at +4°C until being used for disk diffusion assay.^{12,6}

Test Microorganisms

The extracts inhibitory effects were tested against two microbial species including *Escherichia coli*, *Staphylococcus aureus* obtained from Al Assad Hospital, Lattakia. The bacterial cultures of test organisms were maintained on Muller Hinton Agar at 4°C, and were subcultured in petri plates (90mm) prior to use.¹³ DMSO was used as a negative control under the same condition for the tested microorganisms. Imepinam (Primaxin®, 10µg/disc) was used as a positive control. The tests were carried out in duplicate. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the tested microorganisms.¹⁴

Determination of Antimicrobial activity

The antimicrobial activities of the petroleum ether, chloroform, ethyl acetate and methanol extracts by the different concentrations as well as the positive control Imepinam were tested by means of the disk diffusion assay against two human pathogenic bacterial strains, including Gram positive (*S aureus*) and Gram negative (*E coli*).¹⁵

Approximately three cultures from each bacterial stain were inoculated over the surface of a petri plates containing Muller Hinton Agar using sterile swab sticks. Whatman paper disk injected by 20 µl of each concentration and Imepinam disk were placed on the surface of petri plates. The plates were incubated at 37°C for 24 hours.¹⁶ The inhibition zones were calculated by measuring the diameters(mm) of inhibition in (including disk). Experiments were performed in duplicate and the inhibition zones were compared with the positivecontrol.¹⁷

RESULTS

The anti-bacterial activities of leaves extracts were evaluated *in vitro* against *E coli*, *S. aureus*, which are known to cause common infectious diseases. The results

of antibacterial activity were recorded as zone of inhibition in mm around the active extract against the tested microorganisms compared with the standard antibiotic Imepinam used as a positive control.¹⁸

Some *A. maculatum* extracts have shown antibacterial activity against the test microorganisms.

The diameters of zone inhibition of extracts against *S. aureus* were (22-31mm) compared with the diameters of zone inhibition of Imepinam (40-45 mm).

The diameters of zone inhibition of extracts against *E. coli* were (26-31 mm) compared with the diameters of zone inhibition of Imepinam (25-34 mm).

As shown in Table 1, all concentrations of methanolic extract have shown good inhibitory effects against the tested bacteria. While all concentrations of chloroformic extract showed no inhibitory effects against the tested bacteria.

The ether petroleum extracts have shown no inhibitory effects against the test bacteria except at 500mg/ml concentration which have shown inhibitory effects against the test bacteria. However, the ethyl acetate extracts have shown inhibition effects except at 20mg/ml concentration which showed no inhibitory effects.

In conclusion, the methanolic extracts of *A.maculatum* leaves have shown greater antimicrobial activity than the other extracts, followed by ethyl acetate extracts.

Table 1: Antibacterial activity of four *Arum maculatum* leaves extracts

Tested bacteria	chloroformic extracts: mg/ml				
	20	50	100	500	AB
<i>S. aureus</i>	-	-	-	-	45
<i>E. coli</i>	-	-	-	-	25
	petroleum extracts: mg/ml				
	20	50	100	500	AB
<i>S. aureus</i>	-	-	-	22	45
<i>E. coli</i>	-	-	-	26	30
	ethyl acetate extracts: mg/ml				
	20	50	100	500	AB
<i>S. aureus</i>	-	29	30	26	40
<i>E. coli</i>	-	31	30	26	34
	methanolic extracts mg/ml				
	20	50	100	500	AB
<i>S. aureus</i>	26	30	31	27	40
<i>E. coli</i>	29	31	31	30	32

(-) refers to no inhibition

(AB) refers to Imepinam

DISCUSSION

A. maculatum is known to contain alkaloid, saponin, cyanogenic glycosides and monoterpenes. Saponins are a major family of secondary metabolites that occur in a



wide range of plant species. Saponins are steroid and triterpen glycosides so named because of their soap like properties.¹⁹ Most of Terrenes are active against bacteria, fungi, viruses, and protozoa. A terpenoid constituent, capsaicin, has a wide range of biological activities in humans, affecting the nervous cardiovascular, and digestive system, as well as finding use as an analgesic. Monoterpenes and saponins have potent activity against microorganisms as fungi and bacteria.²⁰ The antibacterial properties of saponins are generally ascribed to the ability of these molecules to complex with sterols in bacterial membranes, thus causing pore formation and loss of membrane integrity.²¹ Leaves also contain cyanogenic glycosides which release the known poisonous gas hydrogen cyanide (HCN) or prussic acid. There have been reports of antibacterial properties associated with such glycosides.²² Therefore, the presence of both cyanogenic glycosides and saponins in the leaf extracts of *A. maculatum* is the main reason for the antibacterial activity demonstrated by some leaf extract, because that constituents are dissolved in polar solvents as methanol and ethyl acetate, and these extracts have shown the best antibacterial activity against the tested bacteria.²³

CONCLUSION

The extracts of *A. maculatum* leaves were investigated individually for antimicrobial activity by disc diffusion method against selected species of *Escherichia coli* and *Staphylococcus aureus* to find the inhibitory activities of the microbes. The methanolic extracts followed by ethyl acetate extracts showed considerably good activity than other extracts. These results were compared with standard antibiotic Imepinim. In conclusion, *Arum maculatum* can be supplemented in the treatment of infectious diseases and leaves extracts of the plant may have the potential to be used as antibacterial agents for searching new medicines.

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