

Antibacterial and Antifungal Activities of *Arum maculatum* L. Leaves Extracts

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Abstract

This study was designed to evaluate the antimicrobial activity of three extracts of the leaves of the *Arum maculatum* L. plant belonging to the Aroidae family collected from Kahramanmaraş, Turkey. 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 first prepared with petroleum ether, methanol and ethyl acetate solvents by Soxhlet extraction. The antimicrobial activity of the extracts was then assessed using the agar-well diffusion method against gram-positive, gram-negative bacteria, and yeast and moulds. All extracts of *Arum maculatum* L. showed good activity against some type of bacteria (*Bacillus cereus*, *Micrococcus luteus*, *Pseudomonas phaseolicola*, *Yersinia enterocolitica* and *Enterobacter aerogenes*) and a mold (*Aspergillus niger*). The activity was compared with known antibiotics such as vancomycin, tetracycline and amphotericin B.

Key words: *Arum maculatum*, agar diffusion method, antimicrobial activity

INTRODUCTION

Turkey is an important floristic center internationally because of its geographic location, climate and the presence of nearly 10.000 natural plant species. The characteristics of plants that both inhibit microorganisms and are important for human health have been researched in laboratories since 1926. Traditional medical treatments used in daily life are now being used with empirical methods. Plants synthesis is a vast and diverse assortment of secondary metabolites. Various plants that humans have been using medicinally for hundreds of years can be attributed to secondary metabolites [1,2].

The *Arum* family, Aroidae, which numbers nearly 1.000 members, mostly tropical, and many of them marsh or water plants, is represented in this country by a sole species, *Arum maculatum* L., familiarly known as Lords and Ladies, or Cuckoo-pint. Being a herbaceous perennial, this is a plant which can grow year after year. In herbaceous perennials, such as the buttercup or daisy, 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. In a woody perennial, such as a tree, the above-ground structure survives through the winter, from which new growth springs every spring [3]. *Arum maculatum* is widely grown throughout Turkey. It is popularly known in the Kahramanmaraş region as Tırşik. The leaves of the plant are used commonly by the local people as a vegetable. The roots of the *Arum maculatum* species are used in such treatments as diaphoretics, expectorants, and vermifuges [4].

The *A. maculatum* is known to contain alkaloid, saponin, cyanogenic glycosides [5,6] 2-heptanone, indole, p-cresol, (E)-caryophyllene, a few monoterpenes,

[7,8] lectin [9].and two unidentified sesquiterpenes [10]. 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 soaplike properties [11]. Terpenes or terpenoids 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 [12]. Lectin, purified from edible *Arum maculatum* tuber, was analyzed through SDS-PAGE and studied for its agglutination property using rabbit erythrocytes. The insecticidal activity of the *Arum maculatum* tuber lectin was tested against two economically important sucking pests, *Lipaphis erysimi* and *Aphis craccivora* [9]. The antimicrobial activity of the root of *A. maculatum* was first reported by Uzun et al [13]. *A. maculatum* root extracts showed no significant inhibition zone to the microorganisms tested.

The human body is able to directly absorb different compounds, and due to the low concentration of these compounds, it has transpired that the plant particularly provides effective protection against infection. This protective property, either through the plant itself or by the compounds contained within, also has the effect of prolonging the shelf life of food in which it is used or prevents it being spoiled by microorganisms. Its current use is as a plant additive to other food and its being consumed by the public may be due to both its health-giving properties and taste.

Many works have been conducted with the aim of identifying the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections (both topical

and systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. Antimicrobial compounds from plants may inhibit microbial growth through different mechanisms and may have significant clinical value in the treatment of resistant microbes [14].

This study was aimed at evaluating the in vitro antimicrobial property of three extracts of the leaves of the *Arum maculatum* L.

MATERIALS and METHODS

Collection and identification of plant materials

A. maculatum L. was collected from Kahramanmaraş in January 2006. The voucher specimens were preserved at the Herbarium of the Department of Biology, Faculty of Science and Art, Eskişehir Osmangazi University, Eskişehir, Turkey (ESOGU).

Plant extracts

The aerial parts of the plant material were dried in shade at room temperature and then ground to a fine powder in a mechanic grinder. The powdered plant materials (30g) were then extracted with 250 mL of petroleum ether, 70% methanol and ethyl acetate solvent for 8 h by Soxhlet equipment. Following filtration with Whatman filter paper (No 1), all extracts were concentrated and evaporated to dryness in vacuo at 55 °C by rotary evaporator [15]. The yields from the different extracts were weighed and dissolved in 1mL 100 % dimethyl sulphoxide (DMSO). All extracts were maintained at +4°C until be used for agar well diffusion assay.

Test Microorganisms

The extracts inhibitory effects on a total of 17 microbial species including 10 bacteria, 6 molds and 1 yeast were used as test organisms in this study. *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923 and *Aspergillus niger* ATCC 10949 were obtained from American Type Culture Collection (ATCC, Rock-wille. MD,USA); *Bacillus cereus* NRRL 3711, *Proteus vulgaris* NRRL B-123, *Micrococcus luteus* NRRL B-1018, *Enterobacter aerogenes* NRRL B-3567, *Aspergillus flavus* NRRL 1957, *Aspergillus parasiticus* NRRL 465, *Aspergillus fumigatus* NRRL 163 and *Candida albicans* NRRL Y-12983 from Northern Regional Research Laboratory (NRRL, USDA Peoria, Illionis /USA); *Fusarium graminearum* (wild type), *Fusarium solani* (wild type) from Eskişehir Osmangazi University, Department of Biology Eskişehir/Turkey and *Yersinia enterocolitica* and *Pseudomonas phaseolicola* from Anadolu University, Department of Biology Eskişehir/Turkey. The bacterial and fungal cultures of test organisms were maintained on Nutrient Agar and Malt Extract Agar slants at 4°C, respectively, and were subcultured in petri dishes prior to use. DMSO alone was used as a control under the same condition for tested microorganisms. Vancomycine (Bioanalyse, 30µg/disc)

and tetracycline (Bioanalyse, 30µg/disc) were used as a positive control for bacteria, and Amphotericin B (Sigma) dissolved in DMSO (10µg/well) was used as a positive control for yeast and fungi. The tests were carried out in triplicate. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the test microorganisms.

Determination of antimicrobial activity

The antimicrobial activities of the petroleum ether, methanol and ethyl acetate extracts from the plant sample were evaluated by means of the agar-well diffusion assay [16,17] with some modifications. In short, fifteen milliliters of the specified molten agar (45 °C) were poured into sterile Petri dishes (Ø 90 mm). The cell suspensions containing 108 CFU/ml cells for bacteria, 107 CFU/ml cells for yeasts, and 105 spore/mL of fungi were prepared and evenly spread onto the surface of the agar plates of Mueller-Hinton agar (Oxoid, UK) for bacteria, or Sabouraud dextrose agar (Oxoid, UK) medium for yeasts and fungi using sterile swab sticks. Once the plates were dried aseptically, 10 mm wells were bored using a sterile cork borer. Extracts (100 µl) were placed into the wells and the plates were incubated at 37°C for 24 h for bacterial strains, 48 h for yeasts, and 72 h for fungi at room temperature; tetracycline and vancomycine (30µg/disc) were used as positive controls for bacteria, and amphotericin B (10 µg/well) for yeasts and fungi. The tests were carried out in triplicate. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

RESULTS

The plant material was subjected to an extraction process, with petroleum ether, methanol and ethyl acetate. The yields were 0.2 % for the petroleum ether extract, 0.4 % for the methanol extract, and 0.3 % for the ethyl acetate extract.

As shown in Table 1, 2 the extracts from *A. maculatum* showed antibacterial against some of the tested gram positive bacteria with the diameters of zone inhibition 15 and 42 mm. The most significant result was that the bacteria isolate *B. cereus* was inhibited by all of the plant extracts.

A. maculatum leaves extracts showed antibacterial properties against some of the tested gram negative bacteria with the diameters of zone inhibition 11 and 27 mm. In this study, extracts from the leaves of *A. maculatum* showed antibacterial against some of the tested yeast and fungi, with the diameters of zone inhibition 12 and 14 mm. *A. parasiticus* and *A. fumigatus* were not inhibited by all of the extracts. *A. niger* was the only fungus that was inhibited by the all of the extracts (14mm). The diameter of the inhibition zone around the active extracts was comparable with the standard antibacterial and antifungal antibiotics used as a positive control.

DISCUSSION

During the last ten years the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance (especially multiple) has increased astronomically. The increase in the number of antibiotic resistant bacteria is no longer matched by expansion in the arsenal of agents available to treat infections. Both the literature and ethno botanical records suggest that plants are the sleeping giants of the pharmaceutical industry. They may provide natural sources of antimicrobial drugs that will/or provide novel or lead compounds that may be employed in controlling some infections globally [18].

The most significant result was that the bacteria isolate *B. cereus* was inhibited by all of the plant extracts. Gram-negative bacteria are generally more resistant compared to the Gram-positive bacteria. The lack of activity of tested plant parts against Gram-negative bacteria could be attributed to the greater resistance of these bacteria, due to the presence of an extra outer membrane (polysaccharides, proteins, and phospholipids) in their cell wall acting as a foreign barrier for substances including antibiotics as well as to a different method of preparing extracts from that used by traditional healers, a process which leads to the separation or loss of some active principles. The later is also true for Gram-positive bacteria [19]. Both *Bacillus cereus* and *Staphylococcus aureus* produce toxins causing diarrhoea and vomiting. The illness occurs when people swallow the bacteria or

Table 1. Antibacterial activity of three extracts from *Arum maculatum* L. (well Ø 10 mm)

Tested bacterial strains	Inhibition zone in diameter (mm/ sensitive strains)				
	Petroleum ether	MeOH	Ethyl- acetate	Vancomycine (disc Ø 6mm)	Tetracycline (disc 6mm)
Gram positive					
Bacteria					
<i>Bacillus cereus</i>	30±1.2	20±0.2	42±0.1	24±0.2	30±0.1
<i>Micrococcus luteus</i>	15±0.2	15±0.1	20±0.3	24±0.1	40±0.1
<i>Staphylococcus aureus</i>	15±0.1	-	-	20±0.1	29±0.1
Gram negative					
Bacteria					
<i>Proteus vulgaris</i>	-	13±0.1	14±0.1	-	15±0.1
<i>Enterococcus faecalis</i>	16±0.1	17±0.2	-	19±0.1	12±0.1
<i>Salmonella typhimurium</i>	18±0.1	-	15±0.2	-	14±0.1
<i>Yersinia enterocolitica</i>	14±1.1	11±0.8	14±0.1	20±0.1	29±0.1
<i>Escherichia coli</i>	-	-	27±0.1	-	30±0.1
<i>Enterobacter aerogenes</i>	13±0.5	11±0.4	12±0.1	25±0.1	12±0.1
<i>Pseudomonas phaseolicola</i>	20±0.1	18±0.7	22±0.4	-	-

-: absence of inhibition

Table 2. Antifungal activity of three extracts from *Arum maculatum* L. (well Ø 10 mm)

Tested fungal strains	Inhibition zone in diameter (mm/ sensitive strains)			
	Petroleum ether	MeOH	Ethyl- acetate	Amphotericin B (well Ø 10 mm)
<i>Candida albicans</i>	12±0.1	13±0.9	-	-
<i>Aspergillus flavus</i>	12±0.3	-	-	-
<i>A. parasiticus</i>	-	-	-	-
<i>A. fumigatus</i>	-	-	-	-
<i>A. niger</i>	14±0.7	14±0.1	14±0.2	12±0.1
<i>Fusarium graminearum</i>	12±1.1	-	-	12±0.1
<i>F. solani</i>	12±0.1	-	-	-

-: absence of inhibition

their spores which then multiply and produce toxins in the intestine, or from eating the toxins already produced in the food [19]. *Arum maculatum* root extracts were tested in vitro for antimicrobial activity against tested microorganisms using disc diffusion and microbroth dilution technique. This research showed that ethanol extracts of *Arum maculatum* showed antimicrobial activity against *S. aureus*, *S. epidermidis* and *S. typhi*, but petroleum ether extracts demonstrated no antimicrobial activity. This research showed that *Arum maculatum*, petroleum ether extracts had MIC values of 39.1 µg/mL against *Staphylococcus epidermidis* [13]. The antifungal property of phytochemicals could involve cytosolic hyperacidity, breakage of electrons transport chain, H⁺-ATPase inhibition, channels inhibition, and intracellular enzymes synthesis inhibition [20]. Uzun et al [13] showed that the ethanol and petroleum ether extracts of *Arum maculatum* showed no anticandidal activity against *C. albicans*. In this study, the petroleum ether extract of *Arum maculatum* leaves showed antifungal activity against *A. niger*, *A. flavus*, *F. solani*, *F. graminearum* and *C. albicans*. *A. niger* causes infections of the brain and other organs [21]. The differences observed in the bioactivity assay suggest the susceptibility of these microorganisms to various secondary metabolites present in this *Arum maculatum*. The composition of these secondary metabolites in turn varies according to the species, climatic conditions, and the physiological state of developments of the plants [22].

A. maculatum is known to contain saponin and a few monoterpenes. Monoterpenes and saponins have potent antifungal activity [23,24]. Saponin possesses antifungal activity in vitro with respect to *Candida albicans*, *C. krusei*, *C. tropicalis*, and *S. cerevisia*. In addition to this, activity was especially high against Gram-positive bacteria (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus* and *Enterococcus faecalis*) with *M. arabica* being the species showing the broadest spectrum of action [25,26]. The antifungal properties of saponins are generally ascribed to the ability of these molecules to complex with sterols

in fungal membranes, thus causing pore formation and loss of membrane integrity [27]. Cyanogenic glycosides release the well known poisonous gas hydrogen cyanide (HCN) (11). Hydrocyanic acid (HCN) or prussic acid is generally found in stressed plants and is formed by enzymatic action on compounds called cyanogenetic glucosides (dhurrin) when growth is adversely affected. *A. maculatum* leaves give off prussic acid when injured. There have been reports of antimicrobial properties associated with glucosides [3,28]. However, it is the presence of both glucosides and saponins in the leaf extracts of *Arum maculatum* that is the main reason for the antimicrobial activity demonstrated by the leaf extracts.

Extract from the leaves of *Arum maculatum* has showed significant antibacterial activities and could be used as an antimicrobial agent in new drug therapy.

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