

The Cytotoxicity and the Antimicrobial Activities of *Arum italicum* Miller

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ABSTRACT: In this study, the cytotoxicity potential and antibacterial and antifungal activities of two different extracts of fresh leaves of *Arum italicum* Miller were investigated. The saponin extract and the alkaloid extract were investigated by Brine Shrimp (*Artemia salina* L.) lethality bioassay method for evaluation of cytotoxic effect. Umbelliferone was used as standard, and both of the extracts showed cytotoxic effect by the Brine Shrimp (*Artemia salina* L.) lethality bioassay method. According to the LC₅₀ results, saponin extract was found to be the most active extract, and classifiable as moderate to highly toxic (LC₅₀ 237.1437 mg/mL⁻¹). Although, the alkaloid extract was showed cytotoxic activity lower than Umbelliferone, LC₅₀ 750.1920 mg/mL⁻¹ value was classifiable as mildly cytotoxic. Antibacterial and antifungal activities were tested by the agar well diffusion and tube dilution methods. 6 bacteria and 6 yeasts were used in the methods Meropenem and Fluconazole were the standards of the methods. The saponin extract was found to be effective against *Staphylococcus epidermidis* and *Staphylococcus aureus* compared with Meropenem. Both of the extracts were not found to be effective against fluconazole as antifungal activity point of view. According to the all studied activity results the saponin contents of the fresh leaves of the plant emphasizing the antimicrobial activity. And, both of the extracts have cytotoxic activity potential.

KEYWORDS: *Arum italicum* Miller; saponin; alkaloid; antibacterial activity; antifungal activity;

1. INTRODUCTION

Turkey is an important floristic area because of their climate and geographical location properties on over the World. This region encourages to survive of medicinal plants. Many well-known medicinal plants are available in Turkish flora. That's why traditional folk medicine has wide extended details and recipes since ancient times.

Araceae family is represented in Turkey by 32 taxa, 22 species, 5 subspecies and 12 varieties. There are approximately ten *Arum* species (Araceae) seen in the Turkish flora (1, 2). These species are known as 'yılan yastığı', 'domuz lahanası', 'nivist' etc (3). Although, the fresh leaves contain saponins, alkaloids especially conisine, mucilages, oxalates are also presented in little concentrations. Due to these contents, the fresh plant is very poisonous for human beings and animals. If the plant is eaten, swelling tongue, bloody vomiting and diarrhea may occur (4). This is why, the fresh plant is used externally. The dried roots, leaves or aerial parts of the plant are used in traditional medicine in Turkey (3). Its fresh rhizomes are also used in traditional medicine as anticancer agent (5). Due to the biologically active compounds of *Arum* sp. are also used as natural preservative. This effect is come from antioxidant activity potential of the species. Many phytochemical studies have detected a variety of biologically active secondary metabolites of *Arum italicum* such as saponins, alkaloids, steroidal compounds, neolignan and furan-type lignans (5,6,7). *Arum italicum* is consumed as food in northern part of Turkey as medical purpose such as antihemorrhoidal, expectorant, antirheumatic etc. (3). To the best of our knowledge, the saponin and the alkaloid extracts of *A. italicum* Miller's fresh leaves have not been investigated from the antimicrobial activity perspective. This study aimed to evaluate the antibacterial, antifungal and cytotoxic activities of those extracts of *Arum italicum* Miller.

2. RESULTS and DISCUSSION

This study aimed to evaluate antibacterial, antifungal and cytotoxic activities of saponin and alkaloid extracts of *Arum italicum* Miller.

According to Brine Shrimp (*Artemia salina* L.) lethality bioassay method, crude extracts with LC₅₀ values less than 100 µg mL⁻¹ are considered highly toxic, those with LC₅₀ values between 100 µg mL⁻¹ and 500 µg mL⁻¹, moderately toxic, the ones with LC₅₀ values between 500 µg mL⁻¹ and 1000 µg mL⁻¹, mildly toxic, and those with LC₅₀ values above 1000 µg mL⁻¹ are considered non-toxic (8). The results of Brine Shrimp (*Artemia salina* L.) are shown below in Table 1. The extracts were compared with well-known, active, natural substance which was Umbelliferone which is the standard for cytotoxic activity. The results indicated that the saponin extract had 3 times more cytotoxic activity potential than alkaloid extract, also 1.5 times more active than standard Umbelliferone (LC₅₀: 237,1437; 750,1920; 377,000, respectively). The saponin extract was found to be the most active extract and classifiable as moderate to highly toxic, and the alkaloid extract was showed cytotoxic activity lower than Umbelliferone but it was classifiable as mildly cytotoxic. The Brine Shrimp method results showed that both of the extracts had cytotoxic activity.

Table 1. The results of the Brine Shrimp (*Artemia salina* L.) lethality bioassay method

Tested material	Yield (mg)	ppm	LC ₅₀ (µg mL ⁻¹)
The saponin extract	20.0	1000:100:10	237.1437
The alkaloid extract	14.0	1000:100:10	750.1920
Umbelliferone	-	500:50:5	377.0

3% Sodium chloride solution was used as blind control vial. There was seen no lethality, and LC₅₀ value was N/A

Antibacterial and antifungal activities were tested by the agar well diffusion and tube dilution methods. The reference standard substances were Meropenem and Fluconazole. The antibacterial activity was tested against Meropenem, and the results are shown below in Table 2. The alkaloid extract had no significant effect on any bacteria. The saponin extract of the plant was found to be effective against *Staphylococcus epidermidis* and *Staphylococcus aureus*. Antifungal activity was tested against Fluconazole, and the results are shown below in Table 2. Both of the extracts were not showed wide ranged antifungal activity, but the saponin extract was found to be effective only on *Candida kefyr*. The alkaloid extract was not showed antifungal activity. The activity detected MIC values were identified as 4.34 µg/mL for both bacteria. The MIC value of antifungal activity test was identified as 69.38 µg/mL for *Candida kefyr*.

Table 2. The results of the antimicrobial activity

Bacteria	The Saponin Extract		The Alkaloid Extract		Meropenem		Solvents	
	Zone	MIC	Zone	MIC	Zone	MIC	CHCl ₃	EtOH
	Diameter (mm)	(µg/mL)	Diameter (mm)	(µg/mL)	Diameter (mm)	(µg/mL)		
<i>S. epidermidis</i> ATCC 12228	24	4.34	-	-	22	6.25	-	-
<i>E. coli</i> ATCC 11229	-	-	-	-	18	1.56	-	-
<i>S. aureus</i> 6538 P	24	4.34	-	-	22	6.25	-	-
<i>P. auroginosa</i> ATCC 1539	-	-	-	-	22	1.56	-	-
<i>P. mirabilis</i> ATCC 14153	-	-	-	-	20	3.13	-	-
<i>K. pneumoniae</i> ATCC 4352	-	-	-	-	17	1.56	-	-
Yeasts		Fluconazole						
<i>C. albicans</i> ATCC 10231	-	-	-	-	23	6.25	-	-
<i>C. tropicalis</i> KUEN 1021	-	-	-	-	16	3.13	-	-
<i>C. kefyr</i> KUEN 1012	12	69.38	-	-	20	3.13	-	-
<i>C. krusei</i> ATCC 6258	-	-	-	-	8	50	-	-
<i>C. guilliermondii</i> KUEN 998	-	-	-	-	10	25	-	-
<i>C. glabrata</i> ATCC 90030	-	-	-	-	2.5	> 100	-	-

EtOH: Ethanol; CHCl₃: Chloroform; MIC: Minimum Inhibitory Concentration; -: No Inhibition

These results showed that saponin extract of fresh leaves of *A. italicum* has antibacterial and antifungal activities. In this study results showed that the saponin extract of *A. italicum* has biological activity. The extract needs to be involved further investigations on biological activities point of view. The irresistible resistance of microorganisms to antimicrobial agents makes the treatment of infectious diseases very difficult. Therefore, today, researchers are working to discover natural sources with antimicrobial effects.

3. CONCLUSION

In this study, two different extracts of *A. italicum*, which is known its medicinal potential by Northern and Eastern Turkey habitant, were studied. The extracts were prepared from fresh leaves of the plant. We found that both of the extracts had potential cytotoxic activity. The saponin extract also showed antibacterial and antifungal activity. The cytotoxic activity results suggest that the saponin extract has significant cytotoxic activity. The extract is needed further studies in order to find out the exact cytotoxic activity profile.

4. MATERIALS AND METHODS

4.1. Plant material

Arum italicum Miller (Araceae) was collected from Ordu-Fatsa, Northern Turkey in 2002 (by Dr. Melek Ulusoylu). The plant was identified by Prof. Dr. Ertan Tuzlacı and the voucher specimens were deposited at the herbarium of the Faculty of Pharmacy, Marmara University, MARE 7368.

4.2. Extraction

The fresh leaves (1.2 kg) of the plant were cut into small pieces. Two kinds of extracts were prepared from the fresh leaves of the plant.

Saponin extract: The material (600g) was macerated with petroleum ether for a day. The extracted material was macerated with hot methanol (MeOH) for a day again. The extract was filtered through Whatman No.1 filter paper. The methanolic extract was kept in refrigerator for 2 days. The precipitated part was separated by filtering and the filtrate was evaporated to dryness (20,04 mg).

Alkaloid extract: The material (600g) was macerated with ethanol (EtOH) for a day. The extract was filtered through Whatman No.1 filter paper. The ethanolic extract was evaporated to dryness. The residue was dissolved in HCl 3% until the pH was made to 1. The acidic solution was shaken with CHCl₃ and the organic phase was separated. NH₃ 10% solution was added to the aqueous-acidic solution until the pH was made 10 which was shaken with CHCl₃. The separated CHCl₃ was evaporated to dryness (10).

4.3. Methods

Agar well diffusion [11] and tube dilution methods [12,13] were used to assess the antimicrobial activity of the saponin and alkaloid extracts of *A. italicum*. Mueller Hinton agar and broth media were used to grown the bacterial strains used in this study. Sabouraud Dextrose agar and broth media were used for the yeasts.

The standard bacteria strains were *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 11229, *Staphylococcus aureus* 6538 P, *Pseudomonas aeruginosa* ATCC 1539, *Proteus mirabilis* ATCC 14153, *Klebsiella pneumoniae* ATCC 4352, and yeast strains were *Candida albicans* ATCC 10231, *Candida tropicalis* KUEN 1021, *Candida kefyr* KUEN 1012, *Candida krusei* ATCC 6258, *Candida guilliermondii* KUEN 998, *Candida glabrata* ATCC 90030 used in this study.

4.3.1. Agar well diffusion method

The saponin extract (200 mg) was dissolved in 9 ml EtOH and, the alkaloid extract (43 mg) was dissolved in 2 ml CHCl₃. Both of the dissolved extracts were diluted at 1/10 ratio with sterile distilled water. Thus, the final concentrations of the extract suspensions were 2,22 mg/mL for the saponin extract and, 2.15 mg/mL for the alkaloid extract. Microorganisms were prepared equal to the turbulence of McFarland 0.5 standard, diluted in 1/100 ratio. Preparation method of microorganism suspensions was followed as explained: A few colonies were taken from fresh agar culture (18 hour), placed in physiological saline and

dissolved homogenously. Then, microorganism suspensions were compared with eyes with McFarland 0.5 standard turbidity ($1-2 \times 10^8$ cfu/mL) in saline. The suspensions were prepared at a turbidity equal to McFarland standard turbidity. Microorganism suspensions were finally diluted in 1/100 ratio in saline and spread on the surface of sterile agar plates with sterile swabs.

6 mm diameter wells were cut in the agar plate. 0.1 mL which was taken from stock extract suspensions were put into the wells. The organic solvents which were diluted in 1/10 ratio and Meropenem (50 µg/mL) and Fluconazole (100 µg/mL) were tested as the same manner as control bacteria and yeasts then petri dishes were incubated in the 37 °C overnight. At the end of the incubation period, inhibition zones diameters were measured in mm.

4.3.2. Tube dilution method

Macrodilution test was used for the evaluation of the minimal inhibitory concentrations (MICs) of the extracts. The test identifies the form inhibition zones on the microorganisms. Microorganism suspensions were prepared from the overnight bacteria and yeast culture, equal to the turbulence of Mc Farland 0.5 standard. It was diluted in 1/1000 ratio and adjusted to approximately 1×10^5 cfu/mL. After 48 hours cultured yeast was suspended in 2 mL sterile physiological saline. After washing, the final concentration was adjusted 0.5×10^3 cfu/mL. After shaking, 1 mL was taken from the first tube and put in to the second tube. Thus, concentrations of the extracts were diluted two times beginning from the first to the 8th tube. Equal amounts of the microorganism suspensions were added to each tube. The 9th tube was control tube that only contained the nutrition broth. Meropenem (50 µg/mL) and Fluconazole (100 µg/mL) were tested as the same manner as controls. These tubes were kept in the incubator overnight at 35 °C. After incubation, the growth in each tube was compared with the control tube. The lowest concentration tube that completely inhibited the growth was recorded as the MIC.

4.3.3. The Brine Shrimp (*Artemia salina* L.) Method

The Brine Shrimp (*Artemia salina* L.) lethality bioassay method [9] was used for the potential cytotoxic activity of the extracts. Umbelliferone was used for standard comparison substance. Brine shrimps (*Artemia salina* L.) eggs were placed, for hatching, in a side of a tank divided by a net and containing a 3% (w/v) sodium chloride solution. A light source was placed in the other side of the tank to attract the nauplii. After 48 hour ten nauplii were added to three series of vials containing three different concentrations (10, 100, 1000 ppm) of the extracts. The vials were maintained under light and after 24 hour the number of survivors was counted. The bioassays were carried out in triplicate. 3% Sodium chloride solution was also used as control. The lethal concentration 50% (LC₅₀ value) of the extracts and the standard error were calculated by Probit analysis (14).

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