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Antibacterial Activity and Chemical Composition of *Arum hygrophilum* Boiss Crude Extracts

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Abstract

Plants are an important origin of novel pharmacological compounds. In Jordan, *Arum hygrophilum* Boiss plant is utilized as food and used as traditional medicine for the management of many diseases. This study was designed to evaluate the antibacterial activity and investigate the chemical components of *Arum hygrophilum* Boiss extracts. Water, methanol and ethanol extracts of the plant were prepared and dissolved in Dimethyl sulfoxide to a final stock concentration of 500mg/ml. The antibacterial effect of the extracts was performed using the well diffusion method against the following bacteria: *Staphylococcus aureus, E.coli, Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella pneumonia, Salmonella typhi.* In addition, antifungal activity was studied using *Candida albicans, Candida tropicalis, Candida krusei,* and *Candida rugose.* As compared with the standard inhibition zone of some antibiotics, the water extract exhibited a significant inhibition zone against *P. aeruginosa* (30 mm ± 3.0), whereas methanol extract showed significant inhibition zones against *P. aeruginosa* (21± 2.5 mm) and against *E. faecalis* (15 ± 0.37mm). No antifungal activity was detected in all plant extracts. Chemical compounds identification was carried out applying Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. Test of the extracts revealed the presence of 21 chemical components in the methanol extract, 11 compounds were detected in the water extract and 23 compounds in the ethanol extract. Among these chemicals, alkaloids were detected; two in methanol extract, (3H)-Pyrimidinone and pyrrolidin-2-one and one alkaloid in the water extract, 1H-Imidazole, 2-ethyl-4,5dihydro. The water extract also contained Tert-butyl hydroxy anisole (phenolic compound). Other important components included: Tetrahydropyranis in methanol extract, Butanoic acid in methanol and water extracts, and 2-amino-1,3-propanediol in the water extract. Since the global scheme is now changing towards the use of traditional plant products to control infectious diseases, we believed that the findings in this study are promising, providing a therapeutic potential, and should be further investigated for their medicinal applications.

Keywords: Medicinal plants, Arum *hygrophilumb* Boiss, Antimicrobial activity, Chemical components of plant extracts, Gas Chromatography- Mass Spectrometry analysis (GC-MS).

1. Introduction

Plants are widely used for a variety of purposes in all human cultures; they are utilized as food, culinary spices, medicinal herbs, and cosmetic products. It was announced that about 80% of people in developing countries are using the plants and their extracts as a primary source of healthcare and traditional medical practice (Bodeker *et al.*, 2005). Worldwide, there are about four billion people depending on herbal drugs; some are used for the treatment of chronic diseases such as diabetes, some neurological disorders and cancer (Salim *et al.*, 2008; Lahlou, 2013).

Medicinal plants are an important source for drug discovery; moreover, many newly approved drugs were generated from certain habits of different cultures (Balunas and Kinghorn, 2005). In the field of infectious diseases, 75% of the used drugs are of natural origin; they are in the top 35 worldwide selling ethical drug sales of 2000-2002 (Rates, 2001; Newmann *et al.*, 2003; Balunas and Kinghorn, 2005). In cancer treatment area, 62% of the consumed drugs are of natural origin (Balunas and Kinghorn, 2005).

Despite the progressive increase in the literature on phytochemistry, only a small number of plant species has been screened biologically. There are still a huge number of plants waiting for future investigation, particularly with the development of highly sensitive analytical screening methods (Evans, 2002; Balunas and Kinghorn, 2005).

Jordan is located at the junction of four different biogeographical sectors: the Sudanian or tropical, the Saharo-Arabian, the Irano-Turanean and the Mediterranean (Alali *et al*., 2006; Alali *et al*., 2008; Feinbrun-Dothan,1986; Al Eisawi, 1998); it is known for its various typography and climate. Jordan is rich and biodiverse in its flora and fauna; where 2,500 plant species of about 900 genera and 140 families were documented, it is nearly 20% of the overall flora that are used in tradition medicine (Oran, 2015). In Jordan, a restricted number of plants has been chemically investigated for their biological

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activities (Hudaib and Aburjai, 2007; Abu-Dahab and Afifi, 2007; Quran, 2009).

Araceae plant family (arum family) has 107 genera and over 3700 species that are distributed worldwide (Afifi *et al*., 2017). In Jordan, four arum species were mentioned in the list of flowering plants of Jordan, they are *Arum hygrophilum*, *Arum dioscorides*, *Arum elongatum* and *Arum palaestinum* (Al-Eisawi, 2013). These four species are collectively called Louf. Jordan's Louf is utilized as spices and cooked like leafy crops; it is used in folk medicine to treat cases with cancer, circulatory system, obesity, internal bacterial infection, diabetic symptoms and poisoning problems.

Arum grows naturally in the mountains, rocky places, forests, red soils, alluvial soils, near water canals, and in the upper Jordan Valley. It is also available in many areas including: Ajlun, Jarash, Irbid, Al-Balqa', Wadi-Shua'ib, in addition to Amman (Al-Eisawi, 1998).

Only few reports are available on the chemical and biological characteristics of Arum species (Afifi *et al*., 2017). Extracts of *Arum palaestinum* were found effective against leukemia (k562) and colon cancer (Hatmal*et al*., 2017); fortified *Arum palaestinum* Boiss had blocked the growth of prostatic tumors in mice (Cole *et al*., 2015). Antimicrobial effect of *Arum palaestinum* was also detected by Afifi *et al*. (1997). Antibacterial effect of *Arum maculatum* leave extracts was also detected by Mansour *et al.* (2015). Extract obtained from *A. hygrophilum* revealed some biological activity *in vitro* and *in vivo*. It inhibits the gastrointestinal enzymes involved in the digestion and absorption of carbohydrate and lipid. In addition, it augmented β-cell proliferation in a dose dependent manner (Afifi *et al.*, 2017).

This project was designed to evaluate the antibacterial activity and investigate the chemical composition of *Arum hygrophilum* Boiss stem and leave extracts.

2. Materials and Methods

2.1. Preparation of Plant Extracts

Stems and leaves of *Arum hygrophilum* Boiss were collected from Al- Salt region in Jordan in the flowering period from March to April, 2018. The harvested plant parts (stems and leaves) were thoroughly washed, chopped into bits, dried and grinded using a blender. Aliquots of 10g dry powdered plant material were extracted with 100ml of solvent by refluxing method. The following solvents were used; hot water, methanol and ethanol for 72h at room temperature (except for hot water at 50C°) with continuous shaking at 170rpm. All extracts were filtered through white canvas and filter units of 0.22-0.45µm pore size. Thereafter, solvents were evaporated using rotary evaporator; the crude extracts weighed and dissolved in Dimethyl sulfoxide (DMSO) to a final stock concentration of 500 mg/ml, and kept at 4°C until use.

2.2. Antibacterial and Antifungal Activity of the Extracts

2.2.1. Agar well diffusion method

Antibiotic assay was performed on Petri dishes containing 20 ml of Mueller Hinton (MH) agar. Following the guidelines given by the Clinical and Laboratory Standards Institute (CLSI, 2018), test cultures of *Staphylococcus aureus* (ATCC 29213), *E.coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 51299), *Klebsiella pneumonia, Salmonella typhi, Candida albicans, Candida tropicalis*, *Candida krusei*, and *Candida rugosa* from overnight growth cultures on nutrient agar were taken and suspended in 3 ml of sterile normal saline; that was to achieve the correct inoculums turbidity that matched 0.5 MacFarland standard turbidity in test tubes. Sterile swab sticks were dipped into the inoculum suspension, and the excess fluid was removed by squeezing the swab against the inner sidewall of the test tube. The swabs were used to streak the entire agar surface of each MH plate; the plates were rotated at 90^o angle each time to ensure an uniform distribution of the inoculums over the whole agar plate. The plates were then allowed to dry for two minutes. Four holes with a diameter of 8 mm were punched aseptically in the agar with a sterile tip; then a volume of 150 µL of each arum extract, water, methanol and ethanol and the control Dimethyl sulfoxide (DMSO) solvent were introduced into a well. Petri-dishes were incubated overnight at an optimum temperature of 37^{o}C for 24 hours. Zones of inhibition were scored depending on the activity of antimicrobial compounds in each extract. Experiments were repeated three times and the inhibition zones were recorded as a mean and compared with the positive control.

2.3. Identification of chemical compounds

Chemical screening of the active compounds present in water, ethanol, and methanol extracts was analyzed using GC-MS analysis. Each sample was reconstituted using 1 ml Dichloromethane (DCM) (Aldrich Chemical Co. ltd., USA.) and passed through a glass wool to expel the solid materials. GC-MS system consisted of an HP 5890 series II plus GC and 5972 quadruple mass selective detector (MSD), and Vectra XM24/100i computer workstation was used. Forty microliter of the collection in triplicate was transferred into auto-sampler glass vials having Teflon covers, and the composition was analyzed. The injector temperature was 250°C, the injection mode was Splitless, and the running time was 39min. The dilution rate was 1- 5 for methanol and water extracts, whereas ethanol extract was analyzed without dilution. Identification of components was created using a computer program that utilized a similarity index, match factor or purity between the unknown spectrum and standard library spectra.

2.4. Statistical analysis

Results were summarized as mean ± standard deviation. Independent t-test was used for statistical analysis. Results were considered significant when $P < 0.05$

3. Results

3.1. Antibacterial activity of Arum hygrophilum Boiss extracts

Water and methanol plant extracts displayed some antibacterial activity. Using *Pseudomonas aeruginosa* ATCC 27853, water extract revealed inhibition zone (30.0 ± 3.0 mm), followed by methanol extract (21 ±2.5 mm) (Figure 1 and Table 1). No inhibition zone appeared with ethanol and DMSO (the negative control) (Figure 1). The inhibition zone of water extract against *P. aeruginosa* was

significant as compared with the standard inhibition zone of some antibiotics including Tetracycline (12.0 ±1.0 mm) (p = 0.001), Polymyxins B (13.0 ±1.5 mm) (p = 0.001) and Gentamycin (11.0 ±1.5 mm) (p = 0.001). The inhibition zone of water extract was as much as that of Ciprofloxacin (32 ± 3.0 mm), the difference between them is not significant (P = 0.460) (table 2).

Methanol extract reveled a significant inhibition zone against *P. aeruginosa* in comparison to Tetracycline (p = 0.001), Polymyxins B (p = 0.003) and Gentamycin (p = 0.001). Methanol extract also revealed inhibition zone (15.0 ± 0.37 mm) against *E. Faecalis.*

Ethanol extract showed no inhibitory effects against all the tested microorganisms (Table 1).

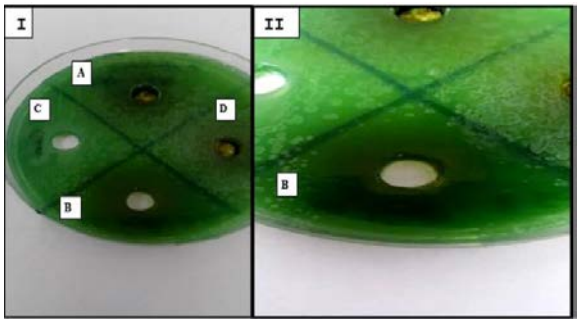

Figure 1.(I) Inhibition zone of *Arum hygrophilum* Boiss on *Pseudomonas aeruginosa* ATCC 27853 using methanol extract (A), water extract (B), ethanol extract (C), and DMSO (D). (II) Inhibition zone of *Arum hygrophilum* Boiss (B) on *Pseudomonas aeruginosa* ATCC 27853 using water extract.

Table 1. Antibacterial activity (zone of inhibition) of different *Arum hygrophilum* Boiss extracts.

Pathogens	Diameter of inhibition zone (mm) mean ± SD		
	Ethanol extract	Methanol extract	Hot water extract
P. aeruginosa	0.0 ± 0.0	21.0 ± 2.5	30.0 ± 3.0
E. coli	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
E. faecalis	0.0 ± 0.0	15.0 ± 0.37	0.0 ± 0.0
S. aureus	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
K. pneumoniae	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
S. typhi	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C. tropicalis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C. krusei	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C. rugosa	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 2. The inhibition zone of water extract of *Arum hygrophilum* Boiss (30 ± 3.0 mm) against *P. aeruginosa* as compared with the standard inhibition zone of some antibiotics

Pseudomonas aeruginosa		
Antibiotics	Inhibition zone (mm) Mean ± SD	P – value
Chloramphenicol (30 µg)	R	
Tetracycline (30 µg)	12 ± 1.0	0.001
Trimethoprim (25 µg)	R	
Erythromycin (15 µg)	R	
Clarithromycin (15 µg)	R	
Penicillin G (10 U)	R	
Polymyxins B (15µg)	13 ± 1.5	0.001
Gentamycin (10 µg)	11 ± 1.5	0.001
Kanamycin (10 µg)	R	
Ciprofloxacin (25 µg)	32 ± 3.0	0.460

3.2. Antifungal activity:

All different Arum extracts were tested against pathogenic *Candida spp*. including; *C. albicans, C. tropicalis*, *C. krusei*, and *C. rugose* (isolated from patients at the Jordan University Hospital). No antifungal activity was detected with different extract.

3.3. Identification of Chemical Components of Arum hygrophilum Boiss extracts

GC-MS chromatography was based on the peak area percentages, retention time, molecular formula and molecular weight. It revealed the presence of 21 chemical compounds in the methanol extract (Table 3), 11 compounds in the water extract (Table 4), and 23 compounds in the ethanol extract (Table 5).

Among the detected chemical components of the extracts, two alkaloids (4(3H)-Pyrimidinone, and Pyrrolidin-2-one) were identified in the methanol extract, and one alkaloid, 1H-Imidazole, 2-ethyl-4,5dihydro in the water extract (Tables 3, 4). The water extract further revealed the presence of phenolic compound, Tert-butyl hydroxy anisole (Table 4).

Table 3: Chemical components of methanol extract of *Arum hygrophilum* Boiss detected by GC-MS

NO.	Name of compounds	Molecular formula	Molecular weight
1	1,2- Butanediol	C4H10O2	90
2	1,2-Dideoxy-1-erythro-pentitol	C5H12O3	120
3	3-Methyl-oxirane-2-carboxylic acid	C5H8O3	116
4	3-(Methoxyethoxymethoxy)-2-methylpentan-1	C10H22O4	154
5	Propane,1,2,3- trimethoxy	C6H14O3	134
6	Butanoic acid, 4-(2-methoxy-1-methyl-2-oxoethoxy)-, methyl ester	C9H16O5	204
7	Decene,3,3,4-timethyl	C13H26	182
8	Borane	C7H18BN	127
9	Tridecane, 3-methylene	C14H28	196
10	Cyclobutane, 2-hexyl- 1,1,4-trimethyl	C13H26	182
11	2-Undecene, 3-methyl	C12H24	168
12	4-Cyclopentene-1,3-dione	C5H4O2	96
13	4(3H)-Pyrimidinone	C4H4N2O	96
14	But-1-ene-3-yne,1-ethoxy	C6H8O	96
15	Pentanoic acid,2-methylphenyl ester	C12H16O2	192
16	Pyrrolidin-2-one	C11H15NO2	193
17	Butibufen	C14H20O2	220
18	Tetrahydropyran	C5H10O	86
19	6- Methyl-cyclohex-2-en-1-ol	C7H12O	112
20	Cyclopentane, 1,2,3-trimethyl	C8H16	112
21	Octane, 4,5-dimethyl	C10H22	142

Table 4. Chemical components of water extract of *Arum hygrophilum* Boiss detected by GC-MS

NO.	Name of compound	Molecular formula	Molecular weight
1	2-Amino-1,3-propanediol	C3H9NO3	91
2	Butanoic acid, 4-chloro	C4H7ClO2	122
3	Cyclohexene, bromo	C6H11Br	162
4	1,2-Ethanediol, monoformate	C3H6O3	90
5	1-Cyclopenten-3-one	C8H10O4	170
6	1-Penten-3-one,2-methyl	C6H10O	98
7	4-Hexen-3-one,2,2-dimethyl	C8H14O	126
8	1H-Imidazole, 2-ethyl-4,5dihydro	C5H10N2	98
9	Glycine, N-cyclopropylcarbonyl-, methyl ester	C7H11NO3	157
10	Tert-butyl-hydroxy anisole	C11H16O2	180
11	Cyclohexane	C6H11N3	125

Table 5. Chemical components of ethanol extract of *Arum hygrophilum* Boiss detected by GC-MS

NO.	Name of compounds	Molecular formula	Molecular weight
1	2,2-Bioxirane	C4H6O2	86
2	1,3-Dioxane,2,4-dimethyle	C6H12O2	116
3	1,3-Dioxane,4-methyl	C5H10O2	102
4	1,3-Dioxepane,2-heptyl	C12H24O2	200
5	Ethanol,2-trimethylsilyl	C5H14O2Si	134
6	Silanol, trimethyl- propanoate	C6H14O2Si	146
7	Silanol, allyldimethyl	C5H12OSi	116
8	3-Hexanol,3-methyl	C7H16O	116
9	2,5-Dimethyl-4-hydroxy-3-hexanone	C8H16O2	144
10	Silane, diethylmethyl	C5H14Si	102
11	2,4-Hexadienal	C6H8O	96
12	2-Hexyne, 4-methyl	C7H12	96
13	2,2-Dimethylcyclopropancarboxylic acid	C8H11NO2	153
14	Methyl-3,3-dimethyl cyclopropane	C9H14O4	186
15	3,3-Dimethylcyclopropane	C7H10O4	158
16	Cyclohexylmethylethylphosphonofluoridate	C9H18FO2P	208
17	Dispiro	C14H22O4	254
18	2-Butenamide	C7H12N2O2	156
19	Phosphonofluoridic acid	C8H16FO2P	194
20	2,2-Dimethyl-3-trans-beta-cyclo-propanecarboxylic acid	C10H16O2	168
21	Eucalyptol	C10H18O	154
22	Benzeneacetaldehyde	C8H8O	120
23	Benzene, propyl	C9H12	120

4. Discussion

Arum hygrophilum Boiss is a well-known plant in Jordan; it is widely consumed as food and used as traditional folk medicine for the management of a variety of diseases. Only few studies are available on the biochemical components and potential medical effect of this plant. This study investigated the antimicrobial activity and chemical components of the plant.

The final stock concentration of the plant extracts (500mg/ml) used in this study was determined on the recommendation of the *Environmental Protection Agency* (*EPA*), being the maximum acceptable total dissolved solids level in drinking water **(**Investopedia, 2019**).**

Antibacterial activity assessment of *Arum hygrophilum* Boiss demonstrated that the water and methanol extracts had a significant antibacterial activity against *P. aeruginosa.* Methanol extract further revealed an inhibitory effect on *E. Faecalis*, whereas ethanol extract displayed no antibacterial influence against all the tested microorganisms. All type of extracts didn't show antifungal activity. However, previous study revealed that ethanol extract of *Arum hygrophilum* displayed an antifungal activity (Khalil and Dababneh, 2007), while petroleum ether, chloroform, ethyl acetate and 70% methanol extracts of *Arum maculatum* manifested greater antimicrobial activities against *E. coli and Staphylococcus aureus* (Mansour *et al*., 2015). It seems that the type of solvent used in preparing the plant extract displays an important role in drawing out the effective ingredients.

The Significant antibacterial effect of *Arum hygrophilum* Boiss against the most resistant bacteria (*P. aeruginosa* and *E. Faecalis*) reveals a promising hope in their eradication.

Fruits and vegetables have antioxidant and scavenging free radical properties; it is due to its polyphenolics, flavonoids and vitamin contents. The GC-MS analysis used in this study revealed the presence of Tert-butyl hydroxy anisole (phenolic compound) in the water extract of *Arum hygrophilum* Boiss; that finding approves its scavenging and antioxidant properties and goes with the reported results of Afifi et al. (2017) on the same plant.

Furthermore, the GC-MS analysis showed the presence of three alkaloids in the *arum hygrophilum* extracts, two in the methanol, (4(3H)-Pyrimidinone and pyrrolidin-2-one) and one in the water extracts (1H-Imidazole, 2-ethyl-4,5dihydro). Alkaloids have a wide variety of physiological effect; they have direct antibacterial, antibiotic enhancing activities, and antivirulence effects (Cushnie *et al*., 2014). The (3H)-Pyrimidinone compound present in the arum extract is commonly used as antibacterial, antifungal, anti-HIV, anticancer, anti-inflammatory, and antiulcer agent (Boukharsa *et al*., 2014), whereas the pyrrolidin-2-one possesses a promising antibacterial, anticancer, and larvicidal potentialities (Suresh *et al*., 2016). The 1H-Imidazole, 2-ethyl-4,5dihydro is one of the Imidazole derivatives; it is used as a base for building up promising antibacterial compounds (Rani *et al*., 2013).

Among the important chemical components of *Arum hygrophilum* methanol extract, Tetrahydropyranis is used as a core in developing non fluoroquinolones compounds; these products exhibited strong antibacterial activity against gram positive bacteria (Surviet *et al*., 2013). Further modification on Tetrahydropyranis, the Dibasic tetrahydropyran-based compounds demonstrated wider antibacterial effects; they are influential against Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (Surviet *et al*., 2017).

Another important chemical compound detected in the methanol and water extracts is Butanoic acid. This chemical structure has various beneficial effects, such as antimicrobial activities, anti-inflammatory and positive effects on animal production, including enhancement of gut development (Bedford and Gong, 2018). Serinol (2-amino-1,3-propanediol) compound present in the water extract is a serine amino acid structural analogue. This compound is usually used in the synthesis of chloramphenicol antibiotic, and has several applications through its derivatives (Andreeßen and Steinbüchel, 2011).

Afifi *et al*. (2017) had identified flavonoids and several plant acids in *Arum hygrophilum* Boiss ethanol extracts. They carried out their investigation through column chromatography and the HPLC-MS. These components are scavenging and antioxidant agents (Amarlal *et al*., 2009; Scalbert *et al*., 2005); and having antimicrobial activities (Cushnie and Lamb, 2005; Daglia, 2012), whereas analysis of ethanol extract of *Arum hygrophilum* Boiss in this study denied the presence of flavonoids and plant acids among the 23 detected chemical components. It seems that the column chromatography and HPLC-MS analysis is more informative than the GC-MS analysis specially if the plant extract involved other component difficult to be detected by GC-MS including some inorganic ions, polymers, nucleotides, and proteins.

More studies are required to monitor the medicinal applications and exploring the toxic effect of *Arum hygrophilum* Boiss and its extracts on vital body organs.

5. Conclusion

In addition to the previously mentioned potentialities of *Arum hygrophilum* Boiss extract, this study demonstrated the importance of the plant components as antibacterial and antioxidants agents used for treatment of various types of infection.

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