

Antibacterial and cytotoxicity evaluation of *Arum hygrophilum* Bioss

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Abstract. – OBJECTIVE: *Arum hygrophilum* Bioss is a plant native to Asia, Europe, and Northern Africa. It is consumed as beverages, spices, or cooked leaves to cure gastrointestinal infections and cancer. This study aims to determine the antibacterial and anticancer effectiveness of *A. hygrophilum* Bioss.

MATERIALS AND METHODS: Using the well-diffusion method, the antimicrobial activity of the plant's aqueous extract and five other organic extracts were evaluated against bacteria often associated with food poisoning. The assessment of the antiproliferative activity by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was done on five cancerous cell lines and on fibroblasts as a reference cell line.

RESULTS: The growth of *L. monocytogenes* was significantly inhibited by the aqueous and the ethanolic extracts. Both extracts had a minimum inhibitory concentration (MIC) of 62.5 mg/mL. The inhibition caused by the methanolic extract had a MIC of 500 mg/mL. The growth of *S. aureus* and MRSA were inhibited by the aqueous extract with a MIC of 500 mg/mL, while the inhibition caused by the ethanolic extract had a MIC of 250 mg/mL on MRSA and 500 mg/mL on *S.aureus*. Both strains of *S.aureus* were also inhibited by the 3-pentanone extract, while the butanol extract only exhibited a moderate growth inhibition against MRSA. The MTT assay showed that the aqueous extract had not affected the proliferation of cancer cell lines. The cytotoxicity of the ethanolic and methanolic extracts had no concentration-inhibition relation-

ship and the IC₅₀ values were above 800 µg/mL for all extracts.

CONCLUSIONS: *L. monocytogenes*, *S. aureus*, and methicillin-resistant *Staphylococcus aureus* (MRSA) were inhibited by different *Arum hygrophilum* Bioss against foodborne pathogens makes it safe to use as a natural food preservative, and as a source for sanitizers and antimicrobials. Further investigation is recommended to determine the cytotoxicity of the plant against additional cancer cell lines.

Key Words:

Arum hygrophilum Bioss, Medicinal plants, Antimicrobials, Foodborne pathogens, Anticancer.

Introduction

Plants are a great source for bioactive compounds that can be used as natural preservatives and for pharmaceutical purposes. These are secondary metabolites, including alkaloids, phenols, flavonoids, tannins, stilbenoids, and anthocyanins¹. The antioxidant activity of these often contributes to their effectiveness to fight infection, cancer, diabetes, aging, cardiovascular, and other degenerative diseases^{2,3}. The World Health Organization (WHO) preserves traditional medicine practices and treatments, and recommends implementation of action plans by governments and international organizations to promote safe and

effective uses⁴. The Center for Disease Control and Prevention highlights that many foodborne pathogens, such as *Listeria monocytogenes*, *Campylobacter*, *Salmonella*, and *Escherichia coli* O157:H7, have developed antibiotic resistance⁵. This demands discovering novel effective antimicrobials especially against the antibiotic-resistance pathogens^{1,5,6}. *Arum* is a wild herbaceous perennial plant that belongs to the Araceae family, and has more than thirty subspecies. Some *Arum* species are frequently used among cancer patients in the Near East countries⁷. The four *Arum* species identified in Jordan: *A. hygrophilum*, *A. palaestinum*, *A. dioscoridis* and *A. elongatum*, are reputed to treat cancer, infection and food poisoning^{8,9}. Extracts and isolated compounds of *A. palaestinum* were reported for their antimicrobial activity. In addition, they were reported for their antiproliferative activity against several cell lines^{10,11} and prostate cancer spheroids in mice¹², and the relaxing effect of uterine muscles isolated from rats and guinea pigs¹³.

A. hygrophilum Boiss is among the twenty subspecies of *A. hygrophilum* reported in Jordan^{9,14}, though it is the least studied. The chemical analysis identified several flavonoids and phenolic acids in the methanolic and ethanolic extracts of *A. hygrophilum*¹⁵⁻¹⁷. Reports reveal that 35.5% of cancer patients in Jordan consume *A. hygrophilum* and *A. palaestinum* as hot beverages, spice, or cooked as a herbal remedy for different types of cancer¹⁸. The aqueous extract of *A. hygrophilum* Boiss had no apparent antiproliferative properties against three colorectal cancer cell lines, but exhibited pancreatic beta-cell line (MIN6) proliferative tendency. It also inhibited the gastrointestinal enzymes involved in the absorption and digestion of lipid and carbohydrates¹⁵. The ethanolic extract of *A. hygrophilum* Boiss exhibited antioxidant properties, and a toxicity to brine shrimp^{16,19}. In a prior study, *A. hygrophilum* Boiss aqueous and methanolic extracts were reported for their growth inhibition of *P. auroginosa*, while the methanolic extract showed a moderate inhibition of *E. faecalis* growth, however, the study indicated no antifungal activity exhibited by the *Arum* extracts against four selected *Candida* species¹⁷.

This study aims to evaluate the antibacterial and antiproliferative properties of *A. hygrophilum* Boiss. The bacteria of choice are pathogens associated with food poisoning and human diseases. The cytotoxicity was evaluated on five cancerous cell lines; breast adenocarcinoma, breast duc-

tal carcinoma, prostate adenocarcinoma, chronic myelogenous leukemia, and skin malignant melanoma. The skin fibroblasts were used as a reference cell line.

Materials and Methods

Plant Collection and Preparation of Extracts

Arum hygrophilum Boiss was collected from Al-Salt region, Jordan, during the flowering period from March-April 2018. The whole plant was thoroughly washed with distilled water, air dried and blended to obtain coarse powder. Six crude extracts were prepared either using hot water or organic solvents of decreasing polarity (methanol, ethanol, n-butanol, 3-pentanone, and chloroform). Each extract was prepared by extraction of 10 g dry plant powder in 100 mL of the desired solvent (2:20 w/v) via the refluxing method for 72 h with continuous stirring either at room temperature or 50°C for the aqueous extract. The extract was filtered through a Whitman filter paper, and then through a 0.45 µm pore size filter. Each extract was concentrated to dryness in a rotary evaporator to give the crude. This crude was refrigerated until use, when it was solubilized in dimethyl sulfoxide (DMSO) to obtain the highest concentration used for the antimicrobial screening. In addition, the antimicrobial effect of the highest concentration of some crudes was evaluated against *L. monocytogenes*, *S. aureus* and its methicillin-resistant strain in the physiological buffer solution (PBS). The highest crude concentration used for the primary screening was 1000 mg/mL. For determining the minimum inhibitory concentration, serial dilutions of the crude in DMSO were prepared immediately before use at 500, 250, 125, 62.5, and 31.25 mg/mL, then filtered. The organic solvents were laboratory grade (Sigma-Aldrich, St. Louis, MA, USA) unless mentioned otherwise.

Screening of the Antibacterial Activity

Agar Well-Diffusion Method

The antimicrobial activity screening was performed by the agar well diffusion method according to guidelines of the Clinical and Laboratory Standards Institute²⁰. Muller-Hinton agar plates were prepared according to the manufacturer instructions (Thermo Fisher Scientific, Waltham,

MA, USA). Wells were made into the agar using a 6 mm diameter sterile borer. The selected bacteria were mainly foodborne pathogens; *Listeria monocytogenes* (ATCC® 7644), *Salmonella typhimurium* [American Type Culture Collection (ATCC® 14028), Manassas, VA, USA], *Staphylococcus aureus* (ATCC® 29213), methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC® BAA-41), vancomycin resistant *Staphylococcus aureus* (VRSA) clinical isolate, *Streptococcus agalactiae* (ATCC® 12386), *Staphylococcus epidermidis* (ATCC® 12228), *Yersinia enterocolitica* (ATCC® 9610), and *Legionella pneumophila* (ATCC® 33152). Bacterial cultures were started in Muller-Hinton (MH) broth, and incubated overnight in the shaker at 37° C. Before use, the inoculum turbidity was standardized at OD₅₂₀ nm = 0.1 (contrasted to 0.5 M McFarland). Bacteria were swabbed uniformly on the agar plates using sterile cotton applicators dipped into the standardized inoculum. Each well had 125 µL of the desired extract concentration, while the control well contained the same volume of DMSO. Plates were then incubated at 37°C for 24 h, and the diameter of the inhibition zones was measured in millimeter (mm). Data are presented as the mean for readings obtained from three different wells for each concentration.

Minimum Inhibitory Concentration (MIC)

If the bacteria was inhibited by the 1000 mg/mL at the primary screening, a determination for the MIC will take place using two-fold lower concentrations starting from 500, 250, 125, 62.5, and 31.25 mg/mL. Five wells were made into the agar before streaking the plates with a standardized bacterial inoculum (see above). Each well was filled with 125 µL of a selected extract or with a control solvent which was one used to solubilize the crude (DMSO or PBS). The plates were then incubated at 37°C for 24 h, and the inhibition zones were measured in mm.

Antibiotics Susceptibility Test

When the 1000 mg/mL, the highest concentration tested, of the selected crude of *A. hygrophilum* Boiss showed inhibition, this inhibition was compared to that caused by standard antibiotics against the same bacterium. The test was performed on MH agar plates per the guidelines of the Clinical and Laboratory Standards Institute²⁰. An overnight bacterial culture was suspended in 3 mL of sterile PBS to obtain an inoculum turbidity equivalent to 0.5 M McFarland stan-

dard turbidity. Using a sterile cotton applicator dipped into the bacterial suspension, the agar plates were streaked with the desired bacteria, dried for a few minutes, and the discs of standard antibiotics were distributed on the agar surface. The Petri dishes were then incubated at 37°C for 24 h before measuring the zones of inhibition in mm. The antibiotics discs used were for cefoperazone (75 µg); chloramphenicol, aztreonam, doxycycline (30 µg); penicillin, ampicillin, streptomycin, imipenem (10 µg); and levofloxacin, ciprofloxacin, methicillin (5 µg). Experiments were done in triplicates, and the average of readings were reported.

Cytotoxicity *in-Vitro* Screening

Cell Lines

The selected cancerous cell lines were: breast adenocarcinoma MCF-7 (ATCC® HTB-22), breast ductal carcinoma T47D (ATCC® HTB-133), skin malignant melanoma A375 (ATCC® CRL-1619), prostate adenocarcinoma PC3 (ATCC® CRL-1435), chronic myelogenous leukemia K562 (ATCC® CCL-243), (ATCC® CRL-1435), chronic myelogenous leukemia K562 (ATCC® CCL-243), and non-cancerous skin fibroblasts MRC-5 (CCD-1064Sk).

Cell Culture

Cells were cultured in Roswell Park Memorial Institute (RPMI) media (EuroClone, Foster City, CA, USA) except for the fibroblast cells which were cultured in Iscove's media. The media were supplemented with 10% fetal bovine serum, 1% of 2.0 mM L-glutamine, penicillin (100 U/mL), and streptomycin (100 µg/mL) (EuroClone, USA). Cells were incubated in 75 cm² culture flasks (Membrane Solutions®, Plano, TX, USA) at 37°C in a NuAire incubator (NuAire, Plymouth, MN, USA) supplemented with 5% CO₂. Upon reaching 80% confluence, cells were treated with the desired crude's concentration, while untreated cells were used as a control and treated with the solvent used to dissolve the crude.

MTT Cell Viability Assays

The MTT assay is an indicator of cell viability, proliferation and cytotoxicity. It is a colorimetric measure of the cellular metabolic activity based on the ability of the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular oxidoreductase enzymes

to reduce the yellow tetrazolium dye, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to form the purple crystals of insoluble formazan^{21,22,23}. Cells were seeded in 96-well culture plates in a final volume of 100 μ L media per well, then plates were incubated in a humidified atmosphere (37°C, 5% CO₂) for 24 h to allow adherence to wells before treatment. The crudes of different extracts were dissolved in DMSO to get the desired concentration. When cells reached confluency, they were treated with the selected crude to obtain final concentrations of 6.25, 12.5, 25, 50, 100, 200, 400, and 800 μ g/mL. Cells were incubated in the humidified atmosphere (37°C, 5% CO₂) for 72 h before performing the MTT assay to determine the cells' viability using the CellTiter non-radioactive cell proliferation assay Kit[®] (Promega, Madison, WI, USA). Each treatment was conducted in triplicate, and the control cells were treated only with DMSO. The optical density for the treated and control wells was measured at 570 nm using a microtiter plate reader (BioTek, Winooski, VT, USA). The half maximal inhibitory concentration (IC₅₀) at which 50% decrease in cells' viability was calculated as a mean value, and the variability was calculated. The cytotoxicity effect was expressed as a percent of the cellular growth inhibition relative to the control sample at 72 h post treatment.

Statistical Analysis

Data were expressed as a mean \pm standard deviation (SD). The statistical significance between different test conditions was determined using the student *t*-test. The difference among groups was significant when $p < 0.05$.

Results

Antimicrobial Activity of *Arum Hygrophilum* Bioss Extract

The aqueous and another five organic extracts of *A. hygrophilum* Bioss (Figure 1 and Table I) inhibited the growth of *L. monocytogenes*, *S. aureus* and *S. aureus* methicillin-resistant strain at 1000 mg/mL which is the highest used concentration of the tested crude. The crudes of aqueous, ethanolic and methanolic extracts dissolved in DMSO showed inhibition only against *L. monocytogenes*, *S. aureus*, and MRSA. The inhibition of these three crudes was equivalent to that shown when they were dissolved in PBS at the same concentration. The n-butanol inhibited the MRSA growth, while the 3-pentanone had a significant inhibition against *S. aureus*, and MRSA. This inhibition was higher than that caused by some standard antibiotics used against these bacteria such as penicillin (10 μ g), ciprofloxacin (5 μ g) and doxycycline (30 μ g) as shown in Table II. There was no apparent effect of all extracts on the Gram-negative bacteria. The chloroform extract had no inhibitory effect on all selected Gram-positive and Gram-negative bacteria.

Minimum Inhibitory Concentration (MIC)

The MIC is the minimum concentration of an extract that inhibits the bacterial growth. It is determined using serial dilutions of the highest concentration of the crude initially inhibited the bacterial growth. The aqueous, ethanolic and methanolic extracts were evaluated for their MIC on the bacteria that they initially inhibited at 1000 mg/mL. The MIC of the aqueous and ethanolic extracts on *L. monocytogenes* was 62.5 mg/mL with inhibition zones of 9.0 mm and 10.0 mm, respec-



Figure 1. Inhibition of *A. hygrophilum* Bioss extracts at 1000 mg/mL on selected bacteria.

Table I. The antibacterial activity of *Arum hygrophilum* Bioss crude at 1000 mg/mL

| | H ₂ O | MeOH | EtOH | n-BuOH | CHCl ₃ | 3-Pentanon |
|--|------------------|------|------|--------|-------------------|------------|
| Gram-Positive Bacteria | | | | | | |
| <i>Listeria monocytogenes</i> ATCC 7644 | 30 | 19 | 30 | 0 | 0 | 0 |
| Vancomycin-resistant <i>Staphylococcus aureus</i> (VRSA)-Clinical strain | 0 | 0 | 0 | 0 | 0 | 0 |
| Methicillin-Resistant <i>Staphylococcus aureus</i> (MRSA) ATCC BAA-41 | 15 | 20 | 20 | 18 | 0 | 22 |
| <i>Staphylococcus aureus</i> ATCC 29213 | 30 | 19 | 30 | 0 | 0 | 20 |
| <i>Staphylococcus epidermidis</i> ATCC 12228 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Streptococcus agalactiae</i> ATCC 12386 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gram-Negative Bacteria | | | | | | |
| <i>Salmonella typhimurium</i> ATCC 14028 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Yersinia enterocolitica</i> ATCC 9610 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Legionella pneumophila</i> ATCC 33152 | 0 | 0 | 0 | 0 | 0 | 0 |

Water=H₂O; Methanol=MeOH; ethanol=EtOH; n-Butanol= BuOH; Chloroform= CHCl₃; 3-Pentanon=C₅H₁₀O.

tively, while the methanolic extract had a MIC at 500 mg/mL (Figure 2). The MICs of these extracts against *S. aureus* strains are shown in Figure 3.

Antibiotics Susceptibility Test

The crude of *A. hygrophilum* Bioss aqueous and ethanol extracts inhibited the growth of *L. monocytogenes*, *S. aureus*, and *S. aureus* BAA-41 (MRSA). The inhibition by 1000 mg/mL was higher or equivalent to some standard antibiotics as shown in Table II.

In vitro Cytotoxicity Assay

The normal skin fibroblasts were used as a reference cell line for verification of cytotoxicity

with a non-detectable antiproliferative IC₅₀ value. The IC₅₀ refers to the concentration that reduces the cells' viability by 50%. It is extrapolated from the dose-response graph by plotting triplicate data points over a concentration range and calculating values. In this study, the MTT assay showed different pattern of viability of treated cells with with the aqueous, ethanolic and methanolic extracts (Figure 4A). The proliferative activity of cells treated with the aqueous extract of *A. hygrophilum* Bioss had no significant inhibition. The ethanolic extract inhibited the cellular proliferation with no consistent pattern among all concentrations. This antiproliferative effect did not exceed 46% at all concentrations, with the

Table II. Antibiotic susceptibility against the bacteria inhibited previously by 1000 mg/mL of *A. hygrophilum* Bioss different extracts.

| | <i>Listeria monocytogenes</i> ATCC 7644 | <i>Staphylococcus aureus</i> | |
|-------------------------|--|------------------------------|------------|
| | | ATCC BAA_41 | ATCC 29213 |
| Cefoperazone (75 µg) | 40 mm | 24 mm | 13 mm |
| Chloramphenicol (30 µg) | 36 mm | 28 mm | 12 mm |
| Aztreonam (30 µg) | 0 | 24 mm | 0 |
| Doxycycline (30 µg) | 13 mm | 0 | 12 mm |
| Ampicillin (10 µg) | 30 mm | 18 mm | 0 |
| Penicillin (10 µg) | 24 mm | 0 | 10 mm |
| Streptomycin (10 µg) | 0 | 15 mm | 12 mm |
| Imipenem (10 µg) | 40 mm | 41 mm | 33 mm |
| Levofloxacin (5 µg) | 38 mm | 30 mm | 17 mm |
| Ciprofloxacin (5 µg) | 24 mm | 26 mm | 10 mm |
| Methicillin (5 µg) | 0 | 0 | 0 |

Measurements of inhibition zones are in millimeter (mm).

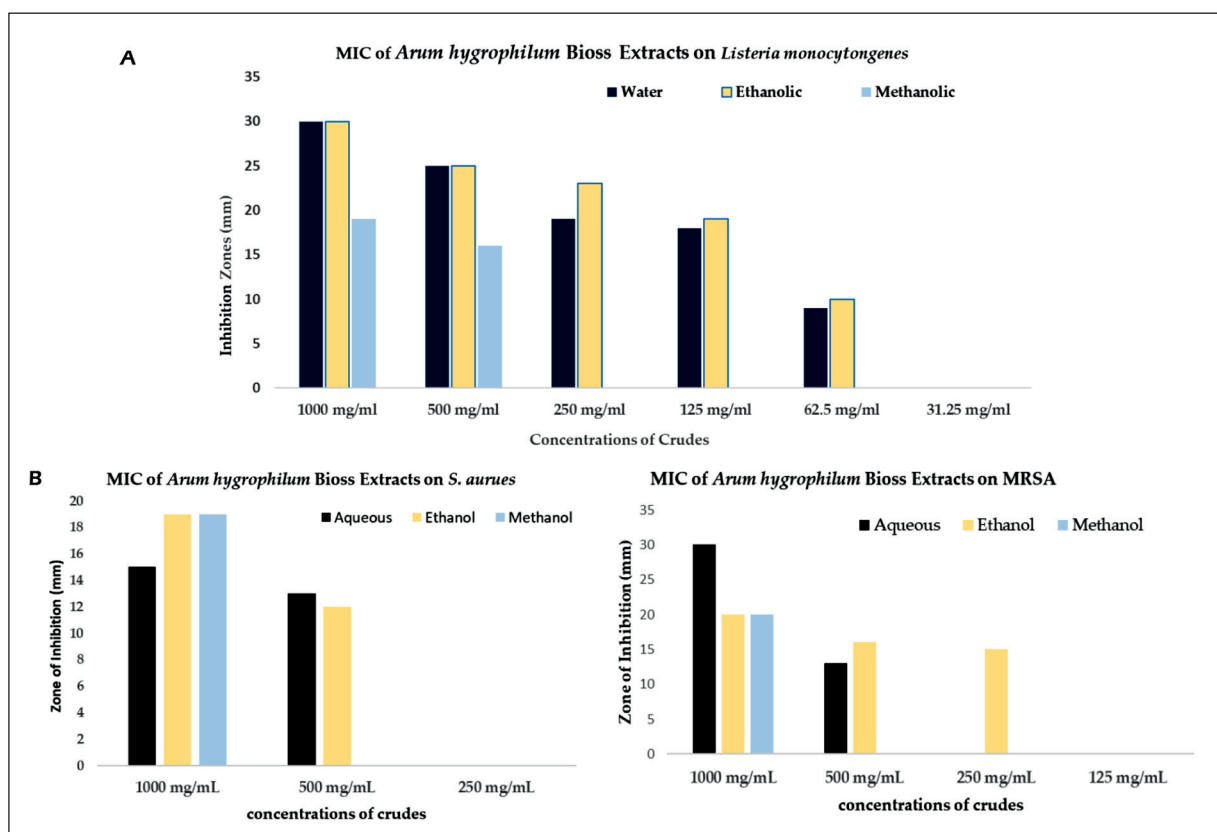


Figure 2. The minimum inhibitory concentrations (MICs) of *A. hygrophilum* Bioss aqueous and ethanolic extracts (mg/mL) against (A) *L. monocytogenes*, (B) *S. aureus* and *S. aureus* BAA-41 (MRSA).

exception of the leukemia cells (K562). At 12.5 $\mu\text{g/mL}$ of the ethanolic crude, the proliferation of K562 was inhibited by 62.9%, noting that the inhibition of the methanolic crude at the same concentration was 31.5% (Figure 4). Both the methanolic and the ethanolic extracts showed no indication that the highest concentration of the crude was more effective in having inhibitory effect on cells' proliferation. The IC_{50} values for the

ethanolic and methanolic crudes were determined to be above 800 $\mu\text{g/mL}$ on all treated cells.

Discussion

Plants, particularly those commonly used in folk medicine, are a promising resource for natural compounds such as flavonoids, alkaloids and

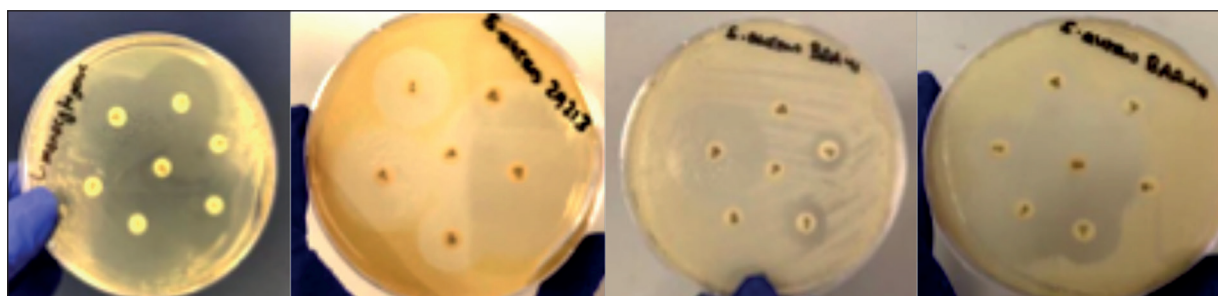


Figure 3. Antibiotic susceptibility against the bacteria inhibited by *A. hygrophilum* Bioss extracts at 1000 mg/mL.

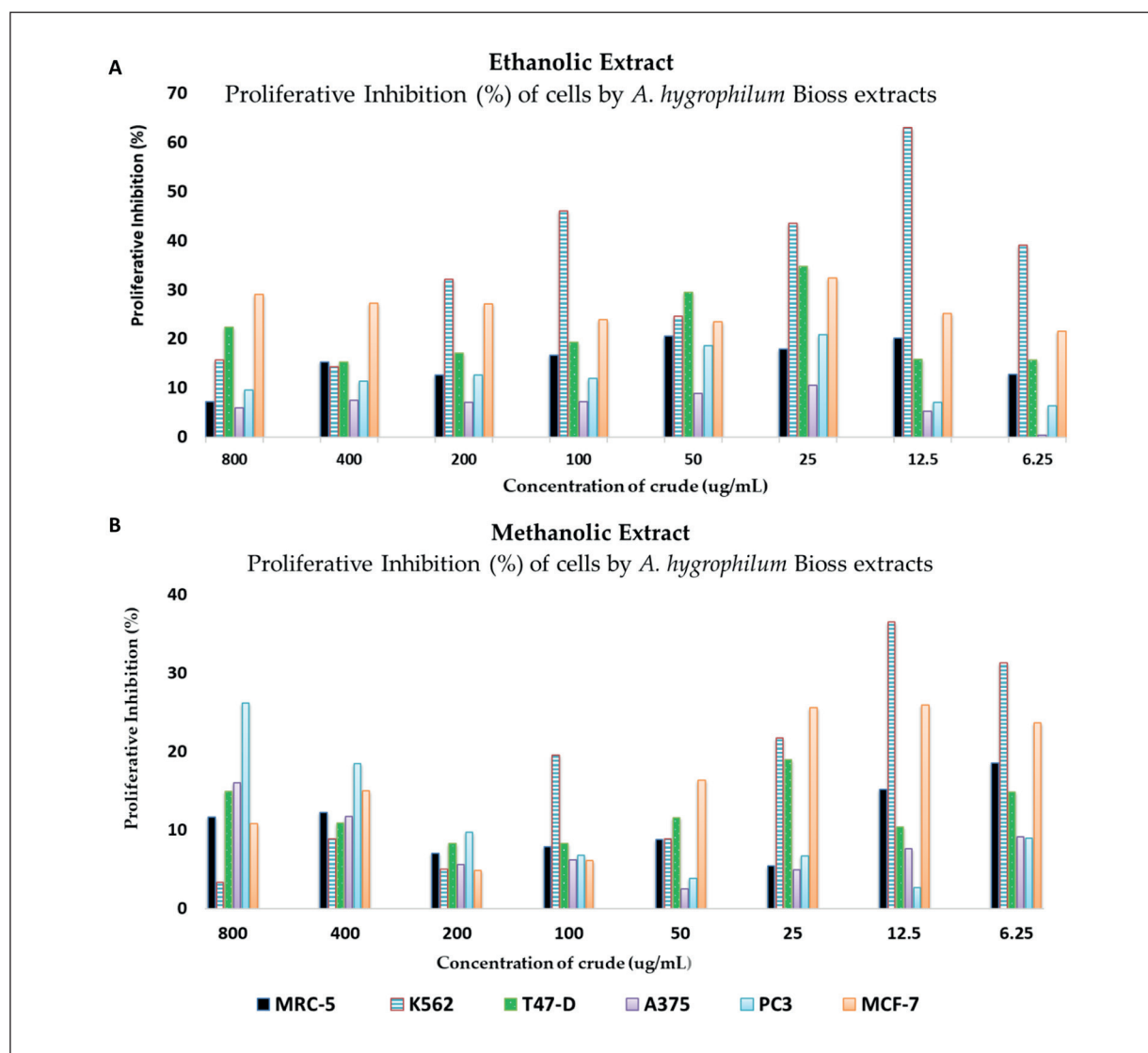


Figure 4. Effect of different concentrations of *Arum hygrophilum* Bioss ethanolic crude (A) and methanolic crude (B) on cells' viability 72 h post treatment. The six cell lines tested were (from left to right); MRC-5 skin fibroblasts, K562 chronic myelogenous leukemia, T47-D breast ductal carcinoma, A375 skin malignant melanoma, PC3 prostate adenocarcinoma, and MCF-7 breast adenocarcinoma.

coumarines. Such compounds have a wide range of biological activities such as antioxidant, anti-spasmodic, antimicrobials and anticancer agents¹. As *Arum hygrophilum* Boiss is a popular medicinal plant mainly consumed as a hot beverage, this study evaluated the effect of the hot aqueous extract on selected bacteria. It was found that the crude of the aqueous extract at 1000 mg/mL only inhibited *L. monocytogenes* which is a Gram-positive foodborne bacteria with a high mortality rate^{2,15}. The bacterial growth was significantly inhibited by crudes of the aqueous and

ethanolic extracts with inhibition zones of 30 mm and MICs of 62.5 mg/mL for both extracts. The inhibition was either equivalent or higher than some antibiotics such as penicillin, ciprofloxacin and doxycycline (Table II).

Studies reported that the growth of *L. monocytogenes* was significantly inhibited by extracts of *A. maculatum* with the lowest reported MIC values for the aqueous and ethanolic extracts²⁴. It is argued that plants' extracts can be vitally used alongside conventional antibiotics in preventing and treating foodborne *Listerial*

diseases. Some plants were reported to be more effective against *Listeria* than some standard antibiotics. Among these plants were kiwi, barberry, Nigella, sage, clove, onions, eucalyptus, mint, *Callistemon citrinus* and cumin²⁵⁻²⁹. The findings of this study bring the attention to *Arum hygrophilum* Boiss as a potential candidate to be used as a natural preservative in the food industry to inhibit foodborne pathogens and maybe to prevent food spoilage. It may also be used as a source for new effective natural antimicrobial agents against *Listeria* species in animal feeds and for human use.

In this study, *A. hygrophilum* Boiss aqueous and ethanolic extracts showed growth inhibition of *S. aureus*, and *S. aureus* BAA-41 (MRSA). The inhibitory effect caused by 1000 mg/mL crude was higher or equivalent to some standard antibiotics such as imipenem (10 µg) used to treat some *Staphylococcus* strains. The MIC values on both *S. aureus* strains by the aqueous crude was 500 µg/mL, and 1000 mg/mL by the methanolic crude. However, the ethanolic extract MIC values was 500 mg/mL on *S. aureus*, and 250 mg/mL on MRSA which is most likely due to the extraction of compounds by ethanol that were not obtained by water.

The bacteria *S. aureus* is one of the most common sources of foodborne diseases in the USA causing gastroenteritis and posing a potential risk for human death. Food Contamination frequently in retail meat, with one or more preformed enterotoxins secreted by *S. aureus* is an important cause of food intoxication³⁰⁻³².

The WHO discusses the strategic plan for food safety including foodborne pathogens³². Among the food safety initiative is the promotion of development of alternative methods for the bacterial inhibition of food or food contact surfaces³². Studies reported that many plants exhibiting antibacterial activity against *S. aureus* have been used as a preservative in the food industry and animal feed. Among plants exhibited bacteriostatic and bactericidal activities against highly susceptible strains of foodborne pathogenic *S. aureus* are: *Punica granatum*, *Syzygium aromaticum*, *Zingiber officinales*, *Thymus vulgaris*, *Cuminum cyminum*, *Achyranthes aspera*, *Cynodon dactylon dactylon*, *Lantana camara*, *Tagetes patula*, and *Rosmarinus officinalis*³³⁻³⁶. It was suggested that a combination of some plant extracts with chemical antibacterials achieved greater bactericidal effects at a low concentration compared to the individual use of synthetic antimicrobial

agents suggesting this approach as an alternative eco-friendly methodology used in sanitization and in food industry³⁶.

This study highlights *Arum hygrophilum* Boiss as a promising source for antimicrobials against *S. aureus* and MRSA strain. This plant can be used individually or in combination with other synthetic agents as a food preservative, and for sanitizing food contact surfaces. The inhibition exhibited by the polar extracts is possibly due to the successful extraction of polar bioactive compounds. Studies reported the identification of structurally diverse chemicals from the ethanolic extract of *A. hygrophilum* Boiss such as flavonoids, alkaloids, and phenolic acids^{15,17}. Alkaloids such as 4(3H)-Pyrimidinone and 2-ethyl-4,5dihydro-1H-Imidazole are commonly used as antibacterial, antifungal, and anticancer agents^{37,38}. The antibacterial activity of alkaloids could be attributed to their ability to form a complex with the bacterial extracellular proteins, and cell wall constituents. Other bioactive compounds identified from *Arum* were tetrahydropyrans which exhibited a strong growth inhibitory effect mainly against Gram-positive bacteria³⁹, and Serinol (2-amino-1,3-propanediol) which is utilized in the synthesis of the antibiotic chloramphenicol⁴⁰, and cyclopentene-1,3-dione which exhibits a slight anticancer activity⁴¹. This study showed no observed effect on Gram-negative bacteria by all extracts of *Arum hygrophilum* Boiss maybe due to the complexity of their cell wall and its content of lipopolysaccharides.

The assessment of the cytotoxicity of *Arum hygrophilum* Boiss was due of its reputation in herbal medicine as a remedy to cure cancer. The plant is commonly consumed as a herbal remedy to fight cancer, though, there is no report yet in the literature about its antitumor activity. Plant-based anticancer compounds have also been identified as novel drugs in chemotherapy because being of less harm on the normal human cells. Among such compounds are alkaloids, diterpenes, diterpenoquinone, purine-based compounds, lactonic sesquiterpene, peptides, cyclic depsipeptide, proteins and macrocyclic polyethers⁴¹⁻⁴³. The *A. palaestinum* extracts were reported for their inhibitory effect on prostate cancer spheroids and for diminishing tumor growth rate in xenografted mice prostate tumors without signs of toxicity to normal cells¹². Extracts of this plant also showed antiproliferative activity against leukemia (K562) and colon cancer (HCT-116) cell lines¹⁰.

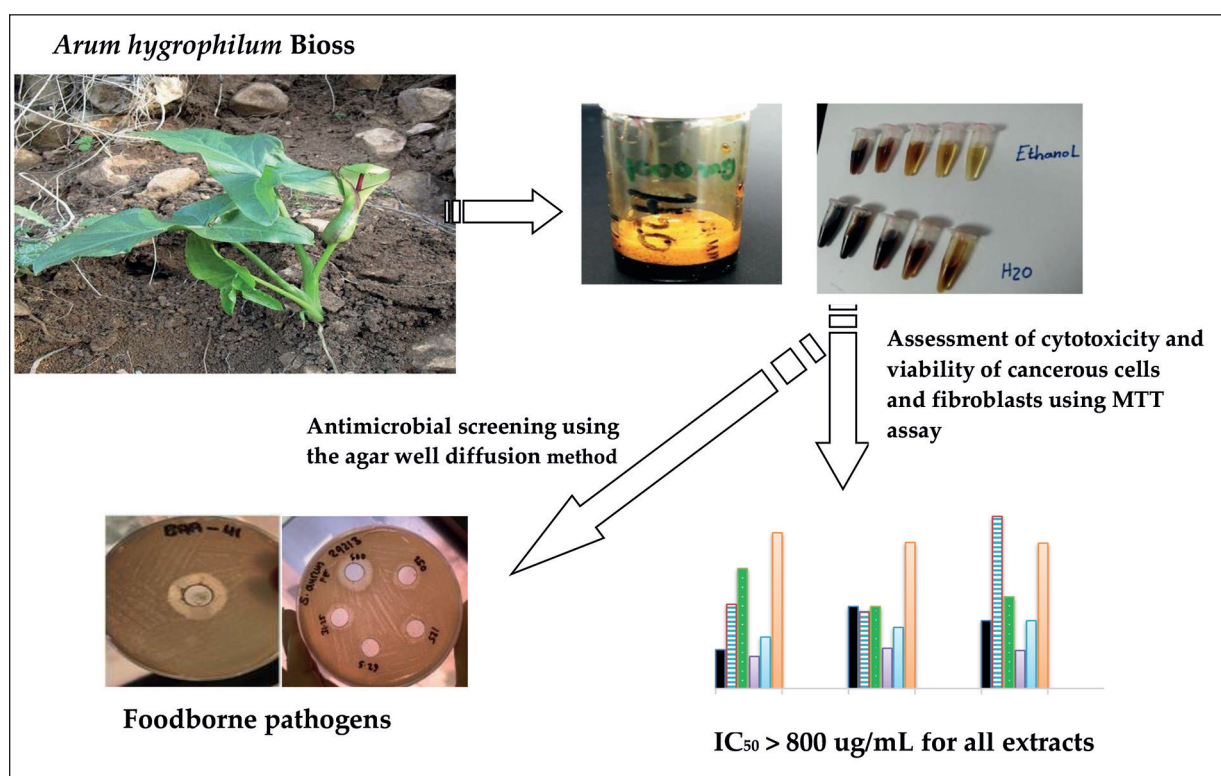


Figure 5. Evaluation of the antimicrobial and cytotoxicity activities of *Arum hygrophilum* Bioss extracts.

This study evaluated the cytotoxicity of *A. hygrophilum* Bioss aqueous, ethanolic and methanolic extracts on five different cancer cell lines; K562, T47D, A375, PC3, and MCF-7 using the MTT assay which determines the cells' viability by quantitative assessment of a metabolic product and indicates the mitochondrial activity in living cells. These factors have a direct relationship with cell proliferation and longevity so the absorbance signal is directly related to the number of alive cells. The MTT assay relies on the conversion of the tetrazolium dye to formazan by mainly mitochondrial succinic dehydrogenases, although some non-mitochondrial enzymes are partially involved such as nicotinamide adenine dinucleotide reductase and flavin oxidase^{21,23}. The fibroblasts used as a non-cancer cell line. The aqueous extract of *A. hygrophilum* Bioss showed no cytotoxic activity on all the cell lines tested, while the cytotoxicity of the ethanolic and methanolic extracts on the different cell lines varied. There was no evidence for a dose-antiproliferative effect relationship for the ethanolic and methanolic extracts on cells. Studies reported that the *in vitro* cytotoxic activity is specific on carcinoma cells when the IC₅₀ is less than 20 µg/mL for a crude, or the IC₅₀ is less than

4 µg/mL for a pure compound within 48 h and 72 h following incubation^{22,44}. In this study, the calculated IC₅₀ values exceeded 800 µg/mL which is the highest tested concentration of the plant's crude indicating that the ethanolic and methanolic extracts exhibited a non-specific cytotoxicity.

Collectively, the results of this study highlight that aqueous extract of *A. hygrophilum* Bioss can be a potential safe source for plant-based food preservatives, sanitizers, and antimicrobials. However, the cytotoxicity findings were not supportive to the use of the plant to cure different types of cancer. There is a need for further investigation on cytotoxicity preferably by using higher concentrations of the plant's extracts, and testing the plant on additional cancer cell lines (Figure 5).

Conclusions

Arum hygrophilum Bioss has a potential to be used in food industry against foodborne pathogens. The cytotoxicity was insignificant against the tested cancer cells so further cytotoxicity studies are recommended. Improvement of the

extraction methodology might increase the effectiveness of the plant's extracts on other pathogens and cell lines.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Authors' Contribution

H.D., L.F.A.: conceptualization, methodology, investigation, supervision, project administration, final editing and review, Y.B., B.A., cytotoxicity design and experimentation, K.H., A.H.N.: draft preparation, M.N., H.S.: interpretation of cytotoxicity data, H.J., N.A.: cytotoxicity data curation. A.N.: Methodology and draft preparation. All authors have read and agreed to the published version of the manuscript.

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