Pharmacological properties of edible *Asparagus acutifolius* and *Asparagus officinalis* collected from North Iraq and Turkey (Hatay)

F.S. Mohammed1*, M. Pehlivan2, E. Sevindik3, H. Akgul4, M. Sevindik5, I. Bozgeyik6 and O. Yumrutas6

1 Department of Biology, Faculty of Science, Zahko University, Duhok, Iraq
2 Department of Medical and Aromatic Plants, Nurdağı Vocational School, Gaziantep University, Gaziantep, Turkey
3 Department of Agricultural Biotechnology, Faculty of Agriculture, Adnan Menderes University, Aydın, Turkey
4 Department of Biology, Faculty of Science, Akdeniz University, Antalya, Turkey
5 Department of Food Processing, Bahçe Vocational School, Osmaniye Korkut Ata University, Osmaniye, Turkey
6 Department of Medical Biology, Faculty of Medicine, Adıyaman University, Adıyaman, Turkey

ABSTRACT

In this study, antioxidant, oxidant, antimicrobial, and antiproliferative activities of *Asparagus acutifolius* L. and *Asparagus officinalis* L., known for their nutritional properties, were determined. In this context, methanol (MeOH) and dichloromethane (DCM) extracts of plants were obtained. Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were determined using Rel Assay kits. Antimicrobial activities of plant extracts were determined against the test microorganisms using the agar dilution method. Antiproliferative activity was tested on the lung cancer cell line A549. As a result of the studies, it has been determined that the plant species have high antioxidant potential. In addition, it was...
observed that the antifungal potentials of plant extracts are high. Antiproliferative activity was determined to be at high level in both plant species. As a result, it has been determined that *A. acutifolius* and *A. officinalis* have medical potential and can be used as natural agents in pharmacological designs.

### 1. INTRODUCTION

Plants are important materials offered by nature. From past to present, plants have been used in line with basic needs such as medicine, food, perfume, spices, clothing, and shelter for humans. Many herbs used in complementary medicine are used in the light of information from the past and in line with supportive scientific research. It carries a significant part of the burden of the health system, especially in developing countries (Yuan et al., 2016). According to the data of the World Health Organization, approximately 80 percent of people in many societies of the world benefit from medicinal plants in the treatment of diseases (WHO, 2019).

Due to the unique phytochemical content of plants, the determination of different bioactivities of different plants always creates an increasing demand and interest for scientists and for sectors such as medicine, pharmacology, and perfumery (Santos-Buelga et al., 2019). In this context, activities of different polarity extracts belonging to *Asparagus acutifolius* and *Asparagus officinalis*, whose antioxidant, antimicrobial, and anti-proliferation properties have not been studied before, were determined. *A. acutifolius* and *A. officinalis* belong to the family Liliaceae. In addition to their nutritional properties, they have considerable medical potential. The genus *Asparagus* is a breed that contains about 150 species. Some of the species belonging to this genus are grown as ornamental plants, while others are grown because of their nutritional properties (Kubota et al., 2012). In our study, antioxidant activities, antimicrobial activities, and antiproliferative effects of *A. acutifolius* and *A. officinalis* plants were investigated. In this context, it is aimed to determine the pharmacological potential of these species known for their nutritional properties.

### 2. MATERIALS AND METHODS

#### 2.1. Collection of plants and laboratory studies

*A. acutifolius* from Hatay (Turkey) and *A. officinalis* from Zahko (North Iraq) regions were collected. The muddy parts of the aerial parts of the collected plant samples were cleaned with the help of distilled water. Then the aerial parts of the plant were subjected to drying under suitable conditions such as humidity, temperature. The dried plant samples were powdered using a mechanical grinder. Extraction with methanol (MeOH) and dichloromethane (DCM) was performed with 250 mL at 50 °C for 6 h using 30 g of powdered plant samples. Crude extracts of plants were obtained by removing solvents (MeOH and DCM) from liquid extracts with a Rotary Evaporator.
2.2. Antioxidant activity tests

Total antioxidant (TAS), total oxidant status (TOS), and oxidative stress index of the plant samples were determined using Rel Assay kits. Trolox (TAS) and hydrogen peroxide (TOS) were used to calibrate the kits. Oxidative stress index (OSI) was determined by proportioning the units of TAS and TOS values to each other (Erel, 2004, 2005).

2.3. Antimicrobial activity tests

Antimicrobial activities of MeOH and DCM extracts of plant samples were determined using the agar dilution method. Concentrations of plant extracts that prevent the growth of microorganisms were determined as MIC (minimal inhibitor concentrations) value. *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Acinetobacter baumannii* ATCC 19606 were used as bacterial strains. *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135 ATCC 13803, and *Candida glabrata* ATCC 90030 were used as yeast strains. Bacterial strains were pre-cultured in Muller Hinton Broth (Merck) and yeast strains were pre-cultured in RPMI 1640 Broth (Sigma-Aldrich). Extracts were obtained using distilled water at concentrations 800 – 12.5 µg mL⁻¹ and tested against microorganisms. Fluconazole, Amphotericin B (yeasts), Amikacin, Ampicillin, and Ciprofloxacin (bacteria) were used as reference drugs (Sevindik, 2020).

2.4. Antiproliferative activity test

The effect of MeOH and DCM extracts of plant samples on A549 cells was determined by the MTT test (3- [4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide). Cells were detached by using 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) to 70–80% binding and planted on plates. The plates were incubated for 24 h. The plant extracts were then diluted to concentrations 25, 50, 100, and 200 µg mL⁻¹ and incubated for 24 h after dilution. After 48 h of incubation, the supernatants were dissolved in the growth medium and replaced with 1 mg mL⁻¹ MTT (Sigma). The plates were then incubated at 37 °C until a purple precipitate was formed. Controls were performed with growth medium not supplemented with FCS (fetal calf serum). Subsequently, supernatants were removed and dissolved by adding dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) to MTT absorbed by cells. The plates were then read at 570 nm using the Epoch spectrophotometer (BioTek Instruments, Winooska, VT) (Bal et al., 2017).

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity

In living organisms, reactive oxygen types are produced as a result of metabolic activities, affecting their environmental. Herbal antioxidants are very important for preventing ROS and reducing oxidative damage. Under normal conditions, especially in eukaryotic systems, ROS is one of the main elements of signal paths. In addition, the antioxidant defence system is activated and suppresses ROS in order to prevent damage to the primary metabolites of living systems at ROS levels that exceed the threshold value. Especially in this defence, many endogenous
antioxidants, especially superoxide dismutase, glutathione peroxidase, ascorbate peroxidase, and catalase, come into play (Chaalal et al., 2019). In cases where endogenous antioxidants are insufficient, it is reported that the supplement antioxidants, which are considered as non-enzymatic supplement antioxidants and evaluated within the natural compound classes, play an important role in suppressing the negative effects of ROS (Sárosi and Bernath, 2008). In this context, TAS, TOS, and OSI values were obtained to determine the antioxidant capacity of A. acutifolius and A. officinalis plants. The results obtained are shown in Table 1.

As a result of our literature review, A. acutifolius and A. officinalis have been reported to have antioxidant activities with analyses such as DPPH, beta-carotene, and reducing power (Nindo et al., 2003; Kasture et al., 2009; Zhao et al., 2012; Di Maro et al., 2013). However, in the literature no antioxidant study in terms of TAS, TOS, and OSI values is found. For this reason, our TAS, TOS, and OSI data are the first report presented for these plants.

Determination of the antioxidant level was done by measuring the increase in TAS. The higher this value is from zero, the higher the antioxidant level. Therefore, as seen in Table 1, TAS values of A. officinalis are higher than those of A. acutifolius. In previous studies on different plant species, TAS, TOS, and OSI values of Allium calocephalum have been reported as 5.853, 16.288, and 0.278, respectively (Mohammed et al., 2019). TAS, TOS, and OSI values of Mentha longifolia subsp. longifolia have been reported as 3.628, 4.046, and 0.112, respectively (Sevindik et al., 2017). TAS, TOS, and OSI values of Rhus coriaria var. zebaria have been reported as 7.342, 5.170, and 0.071, respectively (Mohammed et al., 2018). In another study, TAS values of Calendula officinalis were reported as 5.55 ± 0.41 (Verma et al., 2016). Compared to these studies, it can be stated that TAS value of A. acutifolius is higher than A. calocephalum, M. longifolia subsp. longifolia and C. officinalis and lower than R. coriaria var. zebaria. TAS value of A. officinalis was found to be higher than A. calocephalum, M. longifolia subsp. longifolia, C. officinalis and R. coriaria var. zebaria. Differences occurring between TAS values are probably due to the different plant species and potential of the plant to produce antioxidant compounds.

TOS values refer to all oxidant compounds produced as a result of environmental factors and metabolic activities in the plant. In our study, it was found that A. acutifolius and A. officinalis had high TOS values. A. acutifolius had higher values than M. longifolia subsp. longifolia and R. coriaria var. zebaria and lower than A. calocephalum. A. officinalis had higher TOS values than M. longifolia subsp. longifolia, R. coriaria var. zebaria and A. calocephalum.

OSI value indicates how well the oxidant compounds produced within the plant are neutralised by endogenous antioxidants. The increase in OSI value indicates that the plant tolerates oxidant compounds less and shows that the antioxidant defence system is insufficient to neutralise oxidant compounds. In our study, it was found that A. acutifolius has higher capacity than A. officinalis in terms of OSI value. Compared to different plant species, OSI values of A. acutifolius and A. officinalis were found to be lower than A. calocephalum and higher than M. longifolia subsp. longifolia and R. coriaria var. zebaria. In this context, the antioxidant
defence system of *A. acutifolius* and *A. officinalis* against oxidant compounds was better than *A. calocephalum* and less effective than *M. longifolia* subsp. *longifolia* and *R. coriaria* var. *zebaria*. Due to the high TOS values beside the promising antioxidant levels, additional studies are necessary to recommend the consumption of these plants as food ingredients.

### 3.2. Antimicrobial activity

In recent years, the increase of microorganism-based diseases and the use of wrong drugs against them have led to the development of resistant microorganisms (Sevindik, 2020). In this context, researchers turned to nature to discover new natural antimicrobial agents. Plants are very important materials of the natural ecosystem (Bouarab Chibane et al., 2019). The effects of MeOH and DCM extracts of *A. acutifolius* and *A. officinalis* against bacterium and yeast strains were investigated in our study. The findings are shown in Table 2.

In our study, *A. acutifolius* and *A. officinalis* were found to be effective against the selected test microorganisms at concentrations 50–400 µg mL⁻¹. In addition, it was observed that plants generally had higher antiyeast activities. Previous studies have reported that *A. officinalis* has antimicrobial effects against *Botrytis cinerea*, *Aspergillus luchuensis*, *A. repens*, *C. albicans*, *Cryptococcus albidus*, *Epidermophyton floccosum*, *Microsporum gypseum*, *Mucor racemosus*, *Penicillium italicum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton violaceum*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *Bacillus cereus* (Shimoyamada et al., 1990; Wang and Ng, 2001; Khorasan et al., 2010). In addition, it has been reported that *A. acutifolius* shows antimicrobial effects at different concentrations against *E. coli*, *S. aureus*, *B. cereus*, *Micrococcus luteus*, *Salmonella typhimurium*, *Staphylococcus epidermidis*, *E. faecalis*, *Pseudomonas fluorescens*, *Streptococcus vestibularis*, *Serratia marcescens*, *Saccharomyces cerevisiae*, *C. albicans*, *Candida glabrata*, *Candida utilis*, and *Candida tropicalis* (Çoban et al., 2009). In our study, *A. acutifolius* and *A. officinalis* have been found effective at different concentrations against *S. aureus*, *S. aureus* MRSA, *E. faecalis*, *E. coli*, *P. aeruginosa*, *A. baumannii*, *C. albicans*, *C. krusei*, and *C. glabrata*. It has also been reported that *A. acutifolius* is rich in steroidal saponins, and the saponin-derived compounds have antifungal activity.

| Table 2. Antimicrobial activities of *Asparagus* species |
|----------------|---|---|---|---|---|---|---|---|---|
|               | A  | B  | C  | D  | E  | F  | G  | H  | J  |
| *A. acutifolius* | DCM | 200 | 200 | 100 | 400 | 200 | 400 | 100 | 100 | 100 |
|                | MeOH | 100 | 200 | 100 | 400 | 100 | 400 | 100 | 100 | 50  |
| *A. officinalis* | DCM | 200 | 200 | 200 | 400 | 100 | 400 | 50  | 50  | 50  |
|                | MeOH | 200 | 200 | 100 | 400 | 100 | 200 | 50  | 50  | 50  |
|                | Ampicillin | 1.56 | 3.12 | 1.56 | 3.12 | 3.12 | – | – | – |
|                | Amikacin | – | – | – | 1.56 | 3.12 | 3.12 | – | – | – |
|                | Ciprofloksasin | 1.56 | 3.12 | 1.56 | 3.12 | 3.12 | – | – | – |
|                | Flukanazol | – | – | – | – | – | – | 3.12 | 3.12 | – |
|                | Amfoterisn B | – | – | – | – | – | – | 3.12 | 3.12 | 3.12 |

50, 100, 200, 400: extract concentrations, µg mL⁻¹.

A: *S. aureus*; B: *S. aureus* MRSA; C: *E. faecalis*; D: *E. coli*; E: *P. aeruginosa*; F: *A. baumannii*; G: *C. albicans*; H: *C. krusei*; J: *C. glabrata*.
(Sautour et al., 2007). In our study, higher effectiveness of both plant species against yeasts was found. The antiyeast effect is probably due to the high phenolic content of the plants.

3.3. Antiproliferative effect

Lung cancer, accounting for more than 20%, is the leading cause of cancer deaths among men and women. Every year, more and more people die from lung cancer than colon, breast, and prostate cancers. Although surgery, radiology, and chemotherapy treatments are present for the treatment of cancer, researchers are working tirelessly on complementary approaches. In many parts of the world, people use plants to fight different diseases such as cancer (Arruebo et al., 2011). In this context, the antiproliferative effect of *A. acutifolius* and *A. officinalis* on lung cancer cell line A549 was investigated. The results obtained are shown in Fig. 1.

As seen in Fig. 1, both MeOH and DCM extracts of *A. acutifolius* exhibited higher antiproliferative activity than MeOH and DCM extracts of *A. officinalis*. However, MeOH and DCM extracts from both plant species reduced the viability of A549 cells in a dose-dependent manner. In the literature, there are studies showing the cytotoxic effects of *Asparagus* species on cancer types. Wild *Asparagus laricinus* has been reported to break the cell cycle in HCT-116 colon cancer cells, suppress AKT, ERK, and p70S6K signal pathways, and induce apoptosis (Mfengwana et al., 2019). In the study using methanol extract of *A. laricinus*, it has been reported to show cytotoxic effects and can be used as a chemotherapeutic agent by inducing apoptosis on prostate and breast cancer cells and breaking the cell cycle (Mfengwana et al., 2019). However,
no study on the cytotoxic activity of Asparagus species used in our study on lung cancer cells can be found in the literature. Therefore, our report on *A. acutifolius* and *A. officinalis* is the first showing the results of antiproliferative activity of their different polar extracts. However, additional studies on apoptosis, cell cycle, and signal pathways are required and recommended for more precise understanding of the anticancer activities of these species.

4. CONCLUSIONS

In this study, the biological activities of *A. acutifolius* and *A. officinalis* were determined. As a result of our study, it can be stated that both plant species have significant antioxidant potentials. In addition, it was observed that antiyeast activities of these plant extracts were higher than against the studied bacteria. Antiproliferative effect on lung cancer cell line A549 was determined to be high in both plant species. As a result, *A. acutifolius* and *A. officinalis* can be used as antimicrobial, antioxidant, and anticancer agents in pharmacological designs.

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REFERENCES


