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Effects of Some Herbs on the In-vitro Growth of *Helicobacter pylori* and Their Antioxidant Properties

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ABSTRACT

Helicobacter pylori is one of the important causes of chronic gastritis, peptic ulcer, stomach cancer in humans. The importance of these diseases has led to the development of effective drug treatment regimens against them. The basis of this study is the determination of the effects of some medicinal plants on the in-vitro growth of *H. pylori* and their antimicrobial and antioxidant potential. For this purpose, different parts of 15 plant species were extracted using solvents. Water, ethanol, chloroform, acetone extracts of plants were used and antimicrobial activities of these extracts against both *H. pylori* and other test microorganisms were investigated using the agar disc diffusion methods. The antioxidant properties of the extracts, which were found to be effective in terms of antimicrobial activity, were determined by the thiocyanate method. As a result, acetone extracts of plants; It was determined that it showed higher antimicrobial activity than water, ethanol, chloroform extracts. The most effective two plants against *H. pylori* for water, ethanol, chloroform, acetone were *Capsella bursa-pastoris*, *Acorus calamus*; *Acorus calamus*, *Achillea millefolium*; *Acorus calamus*, *Pimpinella anisum*, *Acorus calamus*, *Achillea millefolium*, respectively. Acetone extracts of *Hypericum perforatum*, *Rosmarinus officinalis*, *Achillea millefolium*; *Acorus calamus*, *Pimpinella anisum* plants were found to have antioxidant properties.

Keywords: *Helicobacter pylori*, In-vitro Studies, Plant extract, Antimicrobial activity, Antioxidant activity.

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Introduction

Helicobacter pylori is gram-negative, highly motile, spiral-shaped bacterium [1, 2]. *H. pylori* has 5 to 7 polar sheathed flagella. These basic characteristics of morphology and motility are thought to be advantageous to these organisms due to their localization in the mucous layer of the gastrointestinal tracts of humans [3, 4]. *H. pylori* colonizes the gastric epithelial surface. *H. pylori* has microaerophilic growth capability and urease activity so the bacterium withstands the stomach's hostile ambiance [4-6]. *H. pylori* is known as the etiologic agent of acute or chronic gastritis and a predisposing factor in peptic ulcer disease, gastric carcinoma [3, 7]. The Natural reservoir of *H. pylori* is humans. The bacterium can be transmitted with the fecal-oral route. Half of the adults in developed countries and 80-90 % of the population in developing countries are infected with these bacteria. *H. pylori* is treated with combined antibiotic therapy [8, 9]. It is known that antibiotic regimens used in *H. pylori* treatment have some side effects and also some of the treated patients develop antibiotic resistance [4, 10, 11]. Due to the various side effects of synthetic drugs used in the treatment of diseases caused by *H. pylori* and other pathogenic bacteria, researches on the use of herbal extracts have intensified in recent years. Herbal extracts are also recommended because they contain natural antioxidant ingredients. Therefore, the fact that a plant material has both antimicrobial and antioxidant effects, it is increased its value even more. There are many plants in the world and in our country that are known to be good for various stomach diseases and are used with traditional methods. This study, it was aimed to determine the antimicrobial effects and antioxidant properties of some medicinal plants in our country, which are used with the belief that they are good for stomach problems, on *H. pylori* which is a stomach pathogen.

Materials and Methods

Plant Material

15 plant samples that have been preferred for gastro-intestinal system disorders by researchers [12, 13] were obtained either as purchased from the local market or collected from the Erzurum region of Turkey. Taxonomic determinations were done using the serial "Flora of Turkey and East Aegean Islands" [12] as well as comparing them with the specimens in the herbarium. Scientific and local names, parts used and folk uses of these plants were summarized in Table 1.

Preparation of Plants Extracts

10 g of powdered parts of the plants (used parts, see Table 1) were separately incubated with 100 ml acetone, chloroform, ethanol, water for 24 hours at room temperature on a shaker (G24 environmental shaker incubator). Final suspensions were filtered using Whatman filter paper (no.1) and extracts were stored at refrigerator until used [5, 14, 15].

Table 1. Plants used in the study

Plant Name (family)	Local name	Part used	Traditional Uses
Achillea millefolium L. (Compositae)	Civanperçemi, Akbaşlı	Flowers, Branches with the leaf	Infection, hemorrhoids, stomach cramp, ulcer, rheumatism
Acorus calamus L. (Araceae)	Eğir kökü, Azakeğeri	Rhizoms	Dysentery, cirrhosis, rachitis, stomach ulcer
Capsella bursa- pastoris L. (Cruciferae)	Çobançantası, Çıngıldaklıot	Flowers, Leaves, Branches	Wounds, bleedings, hemorrhoids, tension, stomach and intestine bleedings
Carum carvi L. (Umbelliferae)	Frenk kimyonu, Karaman kimyonu	Fruits	Cough, stomach and intestine diseases
Foeniculum vulgare Miller (Umbelliferae)	Rezene, Arapsaçı	Fruits	Cough, bronchitis, diarrhea, stomach and intestine pains, wound, tiredness
Glycyrrhiza glabra L. (Leguminosae)	Meyan kökü, Piyan	Roots	Stomach and duodenal ulcer, tuberculosis, gastritis, bronchitis, kidney diseases
Hypericum perforatum L. (Guttiferae)	Sarı kantaron, Binbirdelikotu	Branches with the flower	Asthma, bronchitis, rheumatism, stomach ulcer, tuberculosis, diarrhea, hemorrhoids, antidepressant
Linum usitatissimum L. (Linaceae)	Keten tohumu, Zeyrek tohumu	Seeds	Boil, diabetes, constipation, rheumatism, stomach ulcer, cough, shingles
Matricaria chamomilla var. recutita L. (Compositae)	Mayıs papatyası, Papatya çiçeği	Flowers	Cancer, hemorrhoids, tonsillitis, stomach ulcer, epilepsy, sinusitis, hepatitis, neuralgia, gastritis, enteritis
Melissa officinalis L. (Labiatae)	Oğulotu, Limon nanesi	Leaves	Anemia, asthma, stomach and intestine pains, tension, palpitation of the heart, neurasthenia
Mentha piperita L. (Labiatae)	Kültür nanesi, İngiliz nanesi	Leaves	Stomach ulcer, bronchitis, melancholy, eczema, antiseptic, megrim, liver diseases, epilepsy
Pimpinella anisum L. (Umbelliferae)	Anason, Mesirotu	Fruits	Infection, megrim, angina, gastritis, bronchitis
Rosmarinus officinalis L. (Labiatae)	Biberiye, Kuşdili	Leaves	Antiseptic, hepatitis, asthma, stomach spasm, constipation
Thymus L. (Labiatae)	Kekik, Saterotu	Leaves	Headache, bronchitis, chronic gastritis, stomach ulcer, asthma, cough, antiseptic, bronchitis, rheumatism
Urtica dioica L. (Urticaceae)	Isırganotu, Dızlağan	Leaves	İnfection, ulcer, diabetes, cancer, arthritis, edema, allergy, stomach and intestine diseases, rheumatism, nephrolithiasis, gall bladder, anemia

Antimicrobial Activity

Microorganisms

8 species of bacteria (Helicobacter pylori ATCC 49503, Staphylococcus aureus ATCC 33862, Bacillus subtilis ATCC 6633, Enterobacter cloacae ATCC 13047, Escherichia coli ATCC 25922, Proteus mirabilis ATCC 7002, Pseudomonas aeruginosa ATCC 10145, Klebsiella pneumoniae (Clinic isolate), two species of fungi (Candida albicans ATCC 60193 and Saccharomyces cerevisiae NRRLY 12632) were used in this study. The microorganisms were obtained from the Faculty of Medicine at Karadeniz Technical University, Trabzon, Turkey and the Faculty of Medicine at Atatürk University, Erzurum, Turkey and North University Street, Illinois, USA.

Disc diffusion method

Antimicrobial Activity was carried out by Kirby-Bauer disc diffusion method [15-17]. 100 µl of water, chloroform, ethanol, acetone extracts of 15 plants were transferred onto 6mm diameter antimicrobial susceptibility blank discs (Oxoid). Discs were dried at 37˚C in the incubator [14, 18]. The antibiotics [Ampicilin (10µg /disc), OFX; ofloxacin (10 µg/disc), SCF: sulbactam (30 µg) + cefoperazone (75 µg) = (105µg/disc) and NET: netilmicin (30 µg/disk) for bacteria, NYS::nystatin (30 µg/disk) for fungi] were used as a positive controls. Only solvent-treated discs were used as a negative control. It was taken from the cultures of microorganisms grown in the mediums with inoculating loop and suspended in phosphate- buffered saline (PBS). The dilutions were prepared to be 10^8 CFU/mL according to McFarland turbidity standard no.0.5. These dilutions were used as inoculum [15, 19]. The samples taken from these dilutions using sterile cotton swab sticks were spread over the surface of proper agar plates (Brucella Agar supplemented with %5 human blood for *H. pylori*, Potato Dextrose Agar (PDA) for fungi and Nutrient Agar (NA) for other test bacteria) [20]. Then the absorbed discs were placed on the inoculated agar plates. Brucella Agar plates were incubated under microaerophilic conditions in anaerobic jars with campygen gas generating kit (Oxoid) at 37˚C for 3-5 days [5]. The other plates were incubated at 37˚C for 18-24 hours for bacteria and 3 days for fungi. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone against test microorganisms. Each assay was repeated twice [21].

Minimum inhibitory concentration (Mıc)

It was found that acetone extracts of plants showed stronger antimicrobial activity against *H. pylori* among the extracts of plants prepared using 4 different solvents. Therefore, only the MIC values of the acetone extracts of

the plants were determined. Diameters of the inhibition zone showing the antimicrobial activities of acetone extracts of the plants against *H. pylori* were measured. Then MIC values of the extracts forming the broadest diameter of the inhibition zone against *H. pylori* among acetone extracts of plants were determined. For this purpose, those with diameters of the inhibition zone of 17 mm and higher from acetone extracts of the plants (*Achillea millefolium*, *Pimpinella anisum*, *Rosmarinus officinalis*, *Thymus*, *Hypericum perforatum*) against *H. pylori* were chosen. Powdered parts of the plants were incubated with acetone at room temperature on a shaker. Final suspensions were filtered using Whatman filter paper (no.1). The extracts were evaporated to dryness at 40˚C in a rotary evaporator [5, 22]. MIC values of the extracts were determined by modifying the agar dilution methods. [11, 23, 24]. To determine the MIC values, Brucella Agar, which was sterilized by autoclave, was cooled to 60˚C and 5% human blood was added. Then 24-well plates were placed on a hot plate and maintained at 60 ˚C. In the next step, 1 mL Brucella Agar was added to each well in 24-well plates. On the other hand, 100 mg of the dried extracts were dissolved in 1 mL of Dimethyl sulfoxide (DMSO) and 100 µL of it was taken and diluted with 900 µL of Brucella Agar. Thus, a 10 mg/mL dilution was prepared and the volume of 1 mL from here was transferred to the first well. Then, by transferring the volume of 1 ml from one well to the other, it was provided to prepare two-fold serial dilutions with a concentration of 5 mg/mL in the first well and 0.0024 mg/mL in the 12 well. All tests were done at least in duplicate. The same procedures were applied for ampicillin as positive control and DMSO solution as the negative control. 24-well plate is closed and solidified at room temperature. It was taken from the cultures of *H. pylori* grown in the medium (Brucella Agar supplemented with %5 human blood) with inoculating loop and suspended in phosphate-buffered saline (PBS). The dilution was prepared to be according to 10^8 CFU/mL McFarland turbidity standard no.0.5. This dilution was used as inoculum. 10 µL of this dilution was injected into each well with a micropipette. 24 well-plates were incubated under microaerophilic conditions in anaerobic jars with campygen gas generating kit (Oxoid) at 37˚C for 3-5 days. Microbial growth in each well was determined by observing and comparing wells with the positive and negative controls. The lowest extract concentration in which there was no visible growth was evaluated as MIC [5, 11, 15, 19, 20, 23, 24].

Antioxidant Activity

Thiocyanate method

Since acetone extracts of plants showed stronger antimicrobial activity against *H. pylori*, antioxidant activities of the acetone extract of 5 plants (*Achillea millefolium*, *Pimpinella anisum, Rosmarinus officinalis, Hypericum perforatum*) forming the broadest inhibition zone against *H. pylori* among acetone extracts of plants were determined thiocyanate method.

The antioxidant activities of the extracts were determined according to the thiocyanate method [25]. First, powdered parts of the plants were incubated with acetone at room temperature on a shaker. Final suspensions were filtered using Whatman filter paper (no.1). The extracts were evaporated to dryness at 40˚C in a rotary evaporator [5, 22]. Then, stock solutions of the extracts with a concentration of 1 mg/mL were prepared. The volume of the stock solution corresponding to the desired amounts was placed in the veneer cups with automatic pipettes and the volume was adjusted to 2.5 ml with buffer solution. Then, 2.5 mL of the linoleic acid emulsion was added to each test tube. As a control, a buffer solution (2.5 mL) containing solvent in the amounts used in the experiments (maximum 300 µL) and a mixture of 2.5 mL linoleic acid emulsion was used. Incubation was carried out at 40 °C. At different intervals, 100 µl of each of the samples were taken into test tubes containing 4.7 mL of ethanol, and 100 µL of s Fe^{2+} solution and 100 µL of SCN^- solution were added. The blank sample was obtained by adding 100 µL of Fe^{2+} solution and 100 µL of SCN^- solution to a test tube containing 4.8 mL of ethanol. The absorbances of the samples at 500 nm were read against the blank sample. The incubation was terminated when the control sample reached maximum absorbance. BHT was used as a standard antioxidant in all tests.

Results and Discussion

Antimicrobial Activity

Disc diffusion method

The antimicrobial activities of water, ethanol, chloroform, acetone extracts of 15 plant species obtained by the extraction methods given in the method section were determined against *Helicobacter pylori,* 7 other test bacteria species, 2 fungus species. The antimicrobial activity was evaluated by measuring the diameter of an inhibition zone.

The antimicrobial activities of water extracts of 14 plant species on *H. pylori* and other microorganisms were given in Table 2 and Table 3, respectively. *Linum usitatissimum* wasn't used because water extract of it was not prepared.

Extract of *Capsella bursa-pastoris* plant showed the maximum antimicrobial activity against *H. pylori* in the water extracts and formed diameter of the inhibition zone of 33 mm. This was followed by extracts of *Acorus calamus*, *Glycyrrhiza glabra*, *Achillea millefolium* and *Mentha piperita*, respectively. It was remarkable that water extract of *Capsella bursa-pastoris*, which was effective on *H. pylori*, was also effective on most other test microorganisms (Table 2). This plant extract formed diameter of the inhibition zone of 20 mm against *K. pneumoniae* and 16 mm against *B. subtilis.* Among the water extracts, the plants with the broadest spectrum of antimicrobial activity were *Capsella bursa-pastoris* and *Thymus*. Extracts of these plants showed an inhibition

effect on 6 of the test microorganisms.*C. albicans* was not inhibited by the water extract of any plant. Among the test microorganisms, *H. pylori*, *K. pneumoniae* and *B. subtilis* were found to be the most sensitive bacteria to water extracts (Table 3).

The antimicrobial activities of extracts of ethanol, chloroform, aceton of 15 plant species against *H. pylori* and other microorganisms were given in Table 2 and Table 3, respectively.

The extract belonging to *Acorus calamus* plant showed the maximum antimicrobial activity against *H. pylori* in ethanol extracts and formed diameter of the inhibition zone of 30 mm as can be seen Table 2. This was followed by extracts of *Achillea millefolium*, *Rosmarinus officinalis*, *Thymus*, respectively. When the effect of ethanol extracts on other test microorganisms was examined (Table 3), it was determined that although *Acorus calamus* was the most inhibitory effect against *H. pylori*, it was effective only against *B. subtilis* among other microorganisms. *Achillea millefolium* and *Rosmarinus officinalis* extracts, which were also effective against *H. pylori*, showed an inhibition effect against only 3 microorganism species. Among the ethanol extracts, the plants with the broadest spectrum of antimicrobial activity were *Thymus* and *Foeniculum vulgare* as can be seen Table 3. Extracts of these plants showed an inhibition effect against 7 of the test microorganisms. Among the test microorganisms, the most sensitive microorganisms species to ethanol extracts were respectively *B. subtilis*, *S. aureus*, *K. pneumoniae* whereas *P. aeruginosa* was not affected by any extracts.

Chloroform extract of *Acorus calamus* plant showed the maximum antimicrobial activity against *H. pylori* in chloroform extracts and formed diameter of the inhibition zone of 32 mm. This was followed by *Pimpinella anisum*, *Achillea millefolium* and *Carum carvi* extract respectively (Table 2). The effects of chloroform extracts against other test microorganisms were found different (Table3).

Table 2. Antibacterial activities of extracts of the plant against *H. pylori* ATCC 49503

	Diameter of inhibition zone (mm)			
Plants	**Water**	**Ethanol**	**Chloroform**	**Acetone**
A. calamus L.	21	30	32	37
A. millefolium L.	17	20	21	30
C. bursa-pastoris L.	33	12	10	9
C. carvi L.	8	15	17	15
F. vulgare Miller	—	16	14	9
G. glabra L.	18	10	12	12
H. perforatum L.	—	10	11	19
L. ustitatissimum L.	Not tested	10	16	13
M. chamomilla var. *recutita* L.	—	15	15	13
M. officinalis L.	—	16	16	14
M. piperita L.	15	12	14	14
P. anisum L.	—	16	25	27
R. officinalis L.	—	20	14	24
Thymus L.	10	17	16	20
U. dioica L.	—	9	11	10
Negative Control	—	—	—	—
Ampicillin	45			

Among the chloroform extracts, it was determined that the plant with the broadest spectrum of antimicrobial activity was *Foeniculum vulgare*. This extract formed an inhibition effect against 7 of the test bacteria and also inhibited *C. albicans* fungus species.

Table 3. Antimicrobial activity of extracts of the plant against other test microorganisms [Diameter of inhibition zone (mm)]

	Microorganisms																															
	B. subtilis				***S. aureus***				***E. cloacae***				***E. coli***				***K. pneumoniae***				***P. aeruginosa***				***P. mirabilis***				***C. albicans***				***S. cerevisiae***			
Plants	***W***	***E***	***C***	***A***	***W***	***E***	***C***	***A***	***W***	***E***	***C***	***A***	***W***	***E***	***C***	***A***	***W***	***E***	***C***	***A***	***W***	***E***	***C***	***A***	***W***	***E***	***C***	***A***	***W***	***E***	***C***	***A***
A. calamus L.	13	16	14	18	—	—	9	12	—	—	—	—	—	—	—	—	11	—	—	—	—	—	—	—	—	—	7	—	—	—	—	—	—	—	—
A. millefolium L.	—	15	17	22	8	9	10	12	—	—	—	—	—	—	—	7	—	—	—	—	—	—	—	—	—	8	—	11	—	—	—	—	—	—	—
C. bursa-pastoris L.	16	—	—	—	—	—	7	—	10	—	—	—	9	—	—	—	20	11	10	9	—	—	—	—	9	—	—	—	—	—	—	—	12	—	—	—
C. carvi L.	—	17	17	22	—	11	11	17	—	—	—	—	—	—	—	—	13	9	—	7	—	—	—	—	—	—	10	7	—	—	6	9	—	—	—	—
tF. Vulgare Miller	—	19	18	12	—	21	11	9	—	7	8	—	—	9	12	7	—	7	8	—	—	—	—	—	—	9	12	9	—	7	7	7	—	—	—	—
G. glabra L.	11	11	12	9	—	—	—	—	—	8	—	—	—	—	—	—	13	15	13	14	—	—	—	—	—	—	6	—	—	—	—	—	10	10	9	9
H. perforatum L.	—	15	15	10	—	8	8	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	—	—	—	—	—	8	—	—	—
L. usitatisimum L.	*	11	11	—	*	—	9	—	*	—	—	—	*	—	8	—	*	—	—	—	*	—	—	—	*	—	8	—	*	—	—	—	*	—	—	—
M. chamomilla var. recutita. L.	—	18	18	12	—	11	11	13	—	—	—	—	—	—	6	—	—	—	—	—	—	—	—	—	—	9	8	7	—	—	—	—	—	—	—	—
M. officinalis L.	—	10	—	9	—	—	—	—	—	—	—	—	—	—	—	—	—	14	12	16	—	—	—	—	—	—	—	—	—	—	—	—	—	10	7
M. piperita L.	—	8	—	7	—	—	—	—	—	—	—	—	—	—	—	—	—	15	10	14	—	—	—	—	—	—	—	—	—	—	—	—	__	—	—	—
P. anisum L.	—	19	20	24	—	8	13	15	—	—	—	—	—	7	—	10	—	—	—	11	—	—	—	—	—	8	12	17	—	8	8	12	—	—	—	—
R. officinalis L.	—	15	15	25	—	9	11	13	—	—	—	—	—	—	—	7	10	—	—	—	—	—	—	—	8	—	8	7	7	—	—	—	—	8	—	—	—
Thymus L.	7	14	10	8	10	10	—	11	12	11	—	11	—	—	—	11	12	14	20	14	10	—	—	—	12	14	—	12	—	10	—	—	—	7	9	10
U. dioica L.	—	13	11	6	—	9	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Negative Control	—				—				—				—				—				—				—				—				—			
Antibiotics	28 (OFX)				22 (SCF)				10 (OFX)				18 (Amp.)				12 (OFX)				22 (NET)				13 (Amp.)				18 (Nistatin)				20 (Nistatin)			

—: No inhibition, *: Not tested, W: Water Extract, E: Ethanol Extract, C: Chloroform Extract, A: Acetone Extract

Among the test microorganisms, the most sensitive species to chloroform extracts were respectively *B. subtilis*, *S. aureus* and *K. pneumoni*, Whereas *P. aeruginosa* was not affected by any extracts.

Acetone extracts of *Acorus calamus* plant showed maximum antimicrobial activity in acetone extracts as in chloroform and ethanol extracts of plants and formed diameter of the inhibition zone of 37 mm. This was followed by extracts of *Achillea millefolium*, *Pimpinella anisum*, *Rosmarinus officinalis*, *Thymus*, *Hypericum perforatum*, respectively (Table 2).

When looking at the effect of acetone extracts against other test microorganisms (Table 3), it was determined that *Acorus calamus*, which was the most effective against *H. pylori*, had an inhibition effect against only 3 microorganism species. *Achillea millefolium*, *Pimpinella anisum*, *Rosmarinus officinalis*, *Hypericum perforatum* extracts, which were also effective against *H. pylori*, showed an inhibition effect on 4, 6, 5, 2 microorganism species, respectively. It was determined that *Thymus* was the plant with the broadest spectrum of antimicrobial activity among acetone extracts and this extract formed an inhibition effect against 7 of the test microorganisms. Among the test microorganisms, *B. subtilis*, *K. pneumoniae* and *S. aureus* were found to be the most sensitive species to acetone extracts, respectively. No microorganism was effective against *P. aeruginosa.*

It was determined in our study that acetone extracts of plants had the stronger antimicrobial activity and water extracts had the weaker antimicrobial activity when compared in terms of different solvents. When various research results were examined, it was seen that water extracts generally had low antimicrobial activity compared to other solvents. [17, 26].

However, it is of great importance that the water extracts of a plant exhibit antimicrobial activity since boiled in water or infusion of the plant is generally preferred in the traditional use of medicinal plants. There have been some findings showing that water extracts of some plants have stronger antimicrobial activity than their extracts in various solvents [15, 27-29]

It was determined in our study that water extract of *Capsella bursa-pastoris* was quite effective against both *H. pylori* and other test microorganisms. In this respect, *Capsella bursa-pastoris* was found important for research. Although they weren't as effective against *H.pylori* as *Capsella bursa-pastoris*, water extracts of *Acarus calamus*, *Glycyrrhiza glabra*, *Achillea millefolium* were also effective against *H. pylori*.

Various researchers investigating the antimicrobial effect of plant extracts have used a wide variety of solvents for different parts of different plants [2, 5, 10, 14, 17, 20, 23, 30, 31]. It is not possible to make a preference ranking valid for all plants and plant parts among these solvents. However, ethanol, chloroform and acetone were preferred as solvents in our research considering the working intensity in the literature and generally looking at the positive results.

When the solvents used in our study were evaluated for its potential to extract bioactive substances in the plants, acetone was found to be superior to other solvents. Indeed, regardless of the inhibition zone diameter sizes, in general, 29 of 140 samples (20.71 %) of water extracts, 61 of 150 samples (40.66 %) of ethanol extracts, 59 of 150 samples (39.33%) of chloroform extracts, 64 of 150 samples (42.66%) acetone extracts were also found to have an antimicrobial effect (Table 2 and Table 3). This finding is interesting because there have been few studies researching the conclusion that acetone extracts are superior in antimicrobial activity [26]. This researcher has stated that acetone, methanol, ethanol, water extracts of plants have ranked as acetone> methanol-chloroform-water> methylene dichloride> methanol> ethanol> water in terms of antimicrobial effect. In this study, it was also stated in this study that acetone was preferred as a solvent because it can dissolve hydrophilic and lipophilic structures in plants, can be volatile and can be mixed with water, has low toxicity, and can be mixed with polar and non-polar solvents [26]. However, as stated above, acetone extracts have been often found to be inferior to other extracts in terms of antimicrobial effect. As a matter of the fact, when different research results are examined, it has been determined that extracts of ethanol in some of them [31], extracts of petroleum ether in some of them [17], extracts of chloroform and ethyl ether in other some of them [30] have shown stronger antimicrobial activity. In this case, it is considered that it is not possible to offer an ideal type of solvent for the plant or plant part to be used in terms of antimicrobial effects.

If a generalization was made regardless of the wide of the inhibition zones and the solvent type, it was determined that the plants with the broadest spectrum of antimicrobial activity are *Thymus* and *Foeniculum vulgare*. The broad spectrum of activity of a substance with antimicrobial effect is important in terms of its practical use and evaluation Therefore, in our research, the antimicrobial effects of extracts, which had an inhibition effect against *H. pylori*, were also examined on other test microorganisms. It was found to be promising in this regard the plants whose names were mentioned above.

When the test microorganisms were evaluated in general, *B. subtilis, K. pneumoniae, S. aureus* and *H. pylori* bacteria species were the most sensitive species to extracts of plants. *B. subtilis* and *S. aureus* are Gram-positive, *K. pneumonia* and *H. pylori* are Gram-negative bacteria.it was difficult to comment on whether the extracts were more effective against Gram-positive or Gram-negative bacteria based on these results. Most of the other test bacteria were chosen among Gram-negative bacteria because *H. pylori* was the target organism in this study and it has gram-negative characteristics. Two Gram-positive bacteria and two yeasts were included in this study to facilitate the estimation of the spectrums of antimicrobial activity of the extracts. Many researchers have also preferred similar test microorganisms when they have worked with *H. Pylori* [10, 32, 33]. However, according to the literature, the effect of both various plant extracts and other antimicrobials on Gram-negative bacteria is less than

Gram-positive bacteria due to the less permeability of the Gram-negative cell wall [34]. The fact that the plant extracts used in our study inhibited Gram-negative bacteria; it made us think these extracts contain powerful antimicrobials.

In this study, *Bacillus subtilis* and *Staphylococus aureus* species, which were determined as the most sensitive bacteria to extracts of plants, have identified as sensitive species also according to the results of previous research [34]. The single-layer and more permeable cell wall of Gram-positive bacteria including also S. *aureus* and *B. subtilis*, increases their susceptibility to various antimicrobials. [18]. It was seen that the maximum inhibition zone diameters of extracts of the plants used against *H. pylori* in our study were 33 mm (*Capsella bursa–pastoris*), 30 mm (*Acorus calamus*), 32 mm (*Acorus calamus*) and 37 mm (*Acorus calamus*) for water, ethanol, chloroform, acetone, respectively. In some literature studies investigating the antimicrobial activities of plants against *H.pylori*; 26 mm of Black myrobalan (*Terminalia chebula*) plant [15], 42 mm of bearberry (*Arctostaphylos uva-ursi*) and 17 mm of cowberry (*Vaccinium vitis-idaea*) plants. [27], 13 mm of rose oil [19], 40 mm of *Quercus brantii var.persica* [28] have been found to produce inhibition zone diameters against *H. pylori*. has been found to produce inhibition zone diameters against *H. pylori*. In this case, the inhibition zone diameter sizes obtained in our research are quite high and comparable with the above values.

Diameters of the inhibition zone observed in bacterial species which were found to be sensitive both in our research and in previous studies [21] showed that the antimicrobial activities of the extracts used in our study were at an average level. As a matter of fact, acetone extracts of the plants which caused the broadest inhibition according to the results obtained from our study formed a maximum inhibition zone diameter of 25 mm against *B. subtilis* (*Rosmarinus officinalis*), 17 mm against *S. aureus* (*Carum carvi*), 11 mm against *E. coli* (Thymus), 16 mm against *K. pneumoniae* (*Melisa officinalis*). However, it is difficult to say which plant extract is stronger in terms of antimicrobial activity based on these findings. Because, although diameters of the inhibition zone are used for comparison purposes, methodical differences, solvent quality and small differences in the amount absorbed on the disk lead to big errors in this regard. For this reason, MIC values are used, which allow a better comparison of antimicrobial activity.

There were also two types of fungi among the test microorganisms used in our study. Among water, ethanol, chloroform, acetone extracts of the plants in our study, The number of those showing antimicrobial effects against *C. albicans* and *S. cerevisiae* yeast species were determined respectively as 0,4; 3,2; 3,3; 3,3. The maximum inhibition zone diameter (12 mm) against *C. albicans* was produced by acetone extract of *Pimpinella anisum* and the maximum inhibition zone diameter (12 mm) against *S. cerevisiae* was produced by water extract of *Capsella bursa*. None of the water extracts formed an inhibition zone against *C. albicans*. The inhibition zone diameter sizes determined in our study for *C. albicans* and *S. cerevisiae* have been similar to the inhibition diameter values in studies with these species in the literature [35, 36]. Essentially, a researcher has stated that the antifungal properties of herbal extracts are weak from their antibacterial properties and this is due to the structural difference in the cells. According to this investigator, antimicrobial agents must bind to sterols in the cell membrane to inhibit the eukaryotic fungal cell, whereas such binding is not required for non-sterol-bearing procaryotic bacterial cells [18].

Extracts of the plants used in this study were not effective against *Pseudomonas aeruginosa* bacteria species. This result is not surprising. Because *P. aeruginosa* is a species that has a high potential to gain resistance among bacteria and therefore falls outside the spectrum of effect of many antibiotics [1]. As a parallel with the findings obtained from our research, it has been identified as one of the *P. aeruginosa* resistant species in previous studies on the antimicrobial properties of extracts of various plants [15].

Minimum Inhibitory Concentration (MIC)

MIC values were determined also besides diameter sizes of the inhibition zone caused by extracts of plants against test microorganisms in our study. It was found that acetone extracts of plants showed stronger antimicrobial activity against *H. pylori* among the extracts of plants prepared using 4 different solvents. Then MIC values of the extracts forming the broadest inhibition zone against *H. pylori* among acetone extracts of plants were determined. For this purpose, those with a diameter of inhibition zone of 17 mm and broader from acetone extracts of the plants (*Achillea millefolium*, *Pimpinella anisum*, *Rosmarinus officinalis*, *Thymus*, Hypericum *perforatum*) against *H. pylori* were chosen.

The MIC assays of acetone extracts of the six plants against *H. pylori* were given in Table 4. When Table 4 was examined, it was seen that the MIC values of the acetone extracts of the plants varied between 0.019 to 0.625 mg / mL. On the other hand, it was understood that *Acorus calamus* plant had the best (lowest) MIC value (0.019 mg / mL) among acetone extracts of 6 plants.

The MIC values obtained as a result of our research are close to the MIC values determined for extracts of various plants in the literature. Indeed, Ohsaki et al. [33] has found that plant of *Myroxylon peruiferum* has been effective against *H. pylori* with MIC values of 0.078 mg/mL, Sharifi et al. [28] found that plant of *Quercus brantii var.persica* has been effective against *H. pylori* with MIC values of 0.002 mg/mL. These values are smaller (better) than the MIC values obtained in our research. However, there have been also researchers who have been determined MIC values greater (with less inhibitory effect) than our values. For example, Takashima et al. [32] has determined MIC values of 0.3-85.0 mg/mL against *H. pylori* in his study with *Derris malaccensis*. In this case, we can say that the MIC values obtained from extracts of

plants used in our study are at an average level when compared with the values in the literature.

Table 4. Minimum inhibitory concentrations (MIC) of acetone extracts of some plants against *H. pylori ATCC 49503*

Plants	Diameterofinhibition zone (mm)	MIC (mg/mL)
Acorus calamus L.	36-37	0.019
Achillea millefolium L.	28-30	0.039
Hypericum perforatum L.	17-19	0.625
Pimpinella anisum L.	25-27	0.156
Rosmarinus officinalis L.	22-24	0.156
Thymus L.	18-20	0.312
Ampicillin	38-45	0.00039

Antioxidant Activity

Thiocyanate method

Since acetone extracts of plants showed stronger antimicrobial activity against *H. pylori*, antioxidant activities of the acetone extract of 5 plants (*Achillea millefolium*, *Pimpinella anisum, Rosmarinus officinalis, Hypericum perforatum*) forming the broadest inhibition zone against *H. pylori* among acetone extracts of plants were determined. Thiocyanate method and the results were summarized in Figure 1-5.

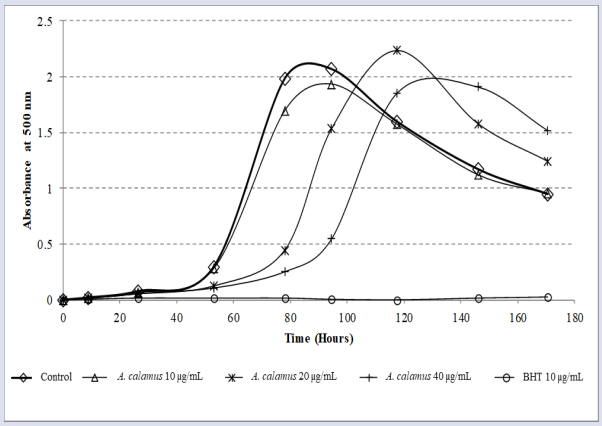

Figure 1. Antioxidant activity of acetone extract of *Acorus calamus*

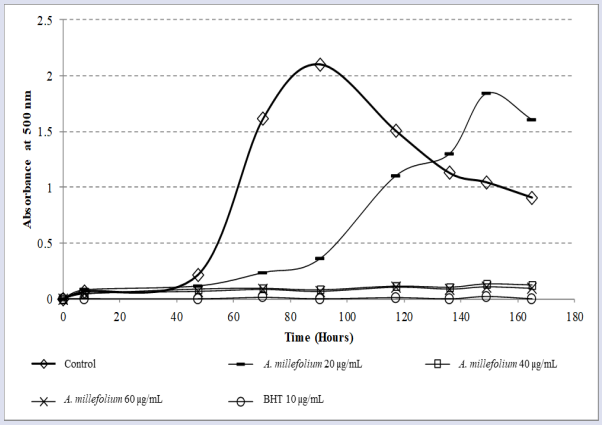

Figure 2. Antioxidant activity of acetone extract of *Achillea millefolium*

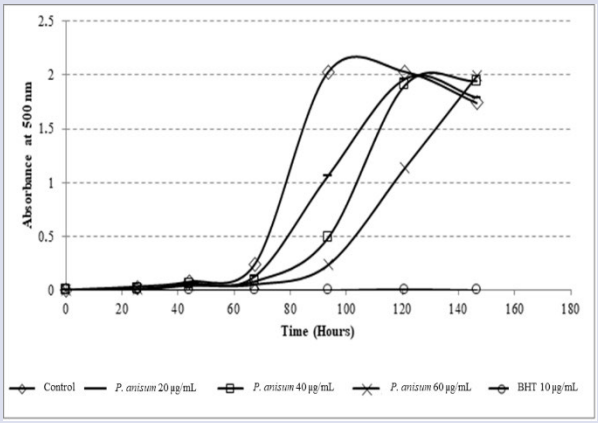

Figure 3. Antioxidant activity of acetone extract of *Pimpinella anisum*

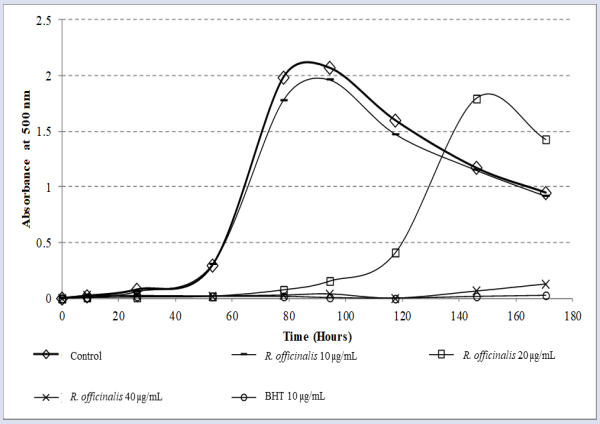

Figure 4. Antioxidant activity of acetone extract of *Rosmarinus officinalis*

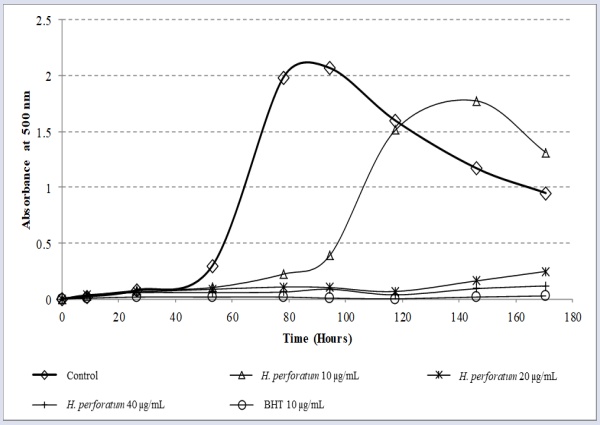

Figure 5. Antioxidant activity of acetone extract of *Hypericum perforatum*

Statistical analyzes of antioxidant activity values were carried out with the SPSS 9.0 package program by performing a two-way analysis of variance. Among aceton extracts of the plants, the extract of *Hypericum perforatum* showed stronger antioxidant activity. This was followed by *Rosmarinus officinalis, Achillea millefolium, Acurus calamus, Pimpinella anisum*, respectively. In all extracts with antioxidant activity, a linear relationship was observed between the concentration of the extract and the antioxidant activity. In other words, as the extract concentration increased, the antioxidant activity increased. The concentration of 40 mg/mL of *Hypericum perforatum* plant showed the maximum antioxidant

activity. This effect of the *Hypericum perforatum* plant followed a parallel course to BHT 50 over for 150 hours as can be seen in Figure 5. It was observed that the concentration of 10 mg/mL of the same plant was ineffective and followed a similar chart to the control. The same was true for other extracts. Generally, a decrease in antioxidant activity was observed in low-concentration extracts around 50-100 hours. On the other hand, in terms of antioxidant activity values of plants, it was determined that the control and extract samples were statistically different from each other ($p<0.05$).

Antioxidants are present in aerobic organisms against undesired oxidation of biomolecules. Antioxidants are substances that prevent or delay oxidation at the initial and/or developmental stages. Therefore, the presence of compounds with antioxidant activity in biological systems is important for life, and many biological functions such as antimutagenic, anticarcinogenic, anti-aging originate from these antioxidants [37, 38].

In recent years, in researches on the antimicrobial properties of plant extracts, taking into account the benefits mentioned above, the antioxidant properties of the studied plant are also included in the study and researches on this subject is gaining intensity [22, 39, 40].

On the other hand, if a herbal product has both antimicrobial and pro-oxidant (oxidation-promoting) qualities, it is certainly not possible to use it in practice. For these reasons, the antioxidant properties of acetone extract of 5 plants (*Hypericum perforatum, Rosmarinus officinalis, Achillea millefolium; Acorus calamus, Pimpinella anisum*) determined to be the most effective in terms of antimicrobial properties in our study were also investigated. For this reason, these 5 plants are recommended for the treatment of diseases due to their antimicrobial properties against *H. pylori* and other test microorganisms and their antioxidant properties mentioned above. Acetone extracts of these 5 plants showed antioxidant properties when Figure 1-5 were examined, it was seen that antioxidant activities of plant extracts increase with increasing concentration. It has been stated that this situation has been due to the increase in the amount of active substance in the extracts with the increasing amount of extract [37]. Since the antioxidant activity in plant extracts is affected by many different factors, it is also stated that it is difficult to determine the main source of antioxidant activity and the contribution of other factors. However, it has been stated that phenolic compounds and flavonoid group substances generally found in various plant extracts have a significant effect on their antioxidant properties [37].

Conclusion

It was determined in our study that the effects of some medicinal plants on the in-vitro growth of *H. pylori* and their antimicrobial and antioxidant potential. As a result, *Hypericum perforatum, Rosmarinus officinalis, Achillea millefolium; Acorus calamus, Pimpinella anisum* plants are recommended for the treatment of diseases due to their antimicrobial properties against *H. pylori* and other test microorganisms and their antioxidant properties. *Acarus calamus* and *Achillea millefolium*, especially should also be tested for in vivo studies. With more detailed studies, it is also necessary to determine the antioxidant properties of other plant extracts (Water, chloroform, ethanol) and to reveal their in-vivo usability.

Conflicts of Interest

The authors state that there is no conflict of interests.

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