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IN- VITRO ANTIMICROBIAL PROPERTIES OF HEDYSARUM ANATOLICUM EXTRACTS

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Abstract:

The genus Hedysarum belongs to the Fabaceae family and is represented by a total of 22 species, 12 of which are endemic in our country. Hedysarum anatolicum is an endemic species that naturally spread in our country. In this study, the antimicrobial effects of methanolic extracts obtained from Hedysarum anatolicum leaves and flowers were tried to be revealed. Eight different concentrations of each extract were used against seven clinically isolated bacterial strains (Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumonia, Burkholderia cepaciae) and a fungus (Candida albicans). The agar well diffusion technique was used to determine the antimicrobial activity of the extracts. As a result, it was determined that both extracts inhibited bacterial growth at different concentrations. Based on the inhibition zone in Streptococcus pyogenes, it was determined that the antibacterial activity of the extract was higher than that of antibiotics on some bacterial species. In conclusion, antimicrobial potential of the extracts obtained from H. anatolicum was revealed with this study.

Keywords: Inhibition zone, Hedysarum, Turkey.

INTRODUCTION

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found in vitro to have antimicrobial properties. [1,2] Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine.[3] Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population.[4] The harmful microorganisms can be controlled with drugs and these results in the emergence of multiple drug-resistant bacteria and it has created alarming clinical situations in the treatment of infections Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Turkey, in terms of existing plant diversity It has a remarkable and rich flora. This wealth; the intersection of three phytogeographic regions Afyon Kocatepe University Journal of Science Sartoria hedysaroides Boiss. & Held. Determination of Antimicrobial Activity of Extracts, Erdoğmuş et al. BATTERY FEBID 12 (2012) 011002 18 being in the region, Southern Europe and South West Being a bridge between Asia, many breeds and Anatolia is the origin and differentiation center of the section. is the result of ecological and phytogeographical differentiation? cause high species endemism (Tan, 1992; Dağcı et al., 2002). Fabaceae is represented by 750 genera and more than 18,000 species. a wide range of species that are widely cultivated and of economic importance. is a dicot family (Ildis, 2001). Sartoria, It is a genus belonging to the Fabaceae family. Sartoria is monotypic endemic of Turkey. is one of the plants. 40-60 cm long pink, perennial, herbaceous and with white, red or yellow flowers It is an economically important plant. It can be used as an ornamental plant in gardens. Fruits formed after flowers 9 mm long and densely hairy. Sartoria of the Animals They are known to eat grass parts. Soil

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helping to prevent erosion while using the soil in terms of minerals provides enrichment (Ertuğrul et al. 2003).

In this study, two different parts of aHedysarum anatolicum plant, leaves and flowers, were analyzed by disc diffusion technique of methanol extracts of 8 different microorganisms. strains (Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumonia, Burkholderia cepaciae) and a fungus (Candida albicans). It was aimed to investigate the antimicrobial activities .

3-Metryl &method

3.1. Microorganisms

3.2 Bacteria

Pathogenic strains of Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,Enterobacter cloacae,Streptococcus pyogenes , Klebsiella pneumonia,Burkholderia cepacia , were obtained from patients specimens of microbiology department in central public health laboratory , and were maintained on Brain Heart Infusion (BHI) agar medium (HiMedia) at 4 ºC for further experiments.

3.3 yeast

like fungi Candida albicans Were isolated and diagnosed depending on the morphological and color characteristics and composition of germ tube with culture characteristics based on (Barnett etal,Halt etal,Baron etal). Yeasts activated in Malt Extract Agar at 25 ° C for four days.

3.3 Plant Sample Collection

The plant materials used in this study consisted of (Hedysarum anatolicum leaves and flowers) . This plant collected from different area in Turkey .

3.4 Bacteria collection

Bacteria collected in Central public health/ Kirkuk, IRAQ

Microorganism	Specimen collection
E.coli	Urin /UTI
Streptococcus pyogenes	Throat swab/ culture
Pseudomonas aeroginosa	Ear swab/ culture
Klebsiella pneumonia	sutum
Burkholderia cepacia	Wound swab
Candida albicans	Vaginal swab / culture
Enterobacter cloacae	UTI

3.5 Preparation of plant extract

Extraction will be made from powdered aHedysarum anatolicum leaves and flowers samples with a soxhlet device. After weighing the plant material, it will be extracted for 6-8 hours. After the resulting mixture is filtered, the solvent will be removed at 40 °C using a Rotary evaporator. The raw extracts obtained will be stored at -20°C in order to prevent loss of activity.

3.6 Preparation of aqueous plant extracts

(1) mg of each aqueous extract was taken and the aqueous extract was dissolved in (9)ml sterile distilled water,The extract was preserved aseptically in a brown bottle at 4c until further use .

3.7 Culture Media

Type of media was required for carrying out this study, Brain Heart Infusion broth, Nutrient agar (Biomark) and Mueller-Hinton agar (Biomark), blood agar base (Oxoid, UK) and MacConkey agar (Oxoid, UK), Mannitol salt agar, Malt Extract Agar.

3.8 Antibiotics drugs

Antibiotics used include: Amoxacılı Clavulanıc acıd , CeftazıdIm,Trımeth0prım sulfa methoxazole,Penicillin,Ampıcıllın,Nitrofuramtion100mg, NalidexicAcid,Amikacin Aztronam Ciprofloxacin,Gentamycin,Ceftaxıme tetracyclın,Chloramphenıcol Imipenem,Cefoxitin,Refadin Cephalothin,Vancomycin .Table (3) shows antibiotics potency.

3-9 Prepratıon of plant extract concentratıons

The stock solutıon was prepared by adding 1ml of it to 9ml of sterile disitlled water the final concentration becomes (1:10) 0.1 % and under sterilization conditions the following concentrations were prepared (1:20),(1:30) (1:40) (1:50) (1:60) (1:70) (1:80)

3-10 Preparation of stock culture

According to Jayaraman et al., stock cultures were maintained at 40 C on nutrient agar slants for bacteria. Active cultures for experiments were prepared by transferring a loopful of culture to 5 ml of Brain Heart Infusion broth and incubated at 37 o C for 24 hours.

3.11 Bacterial isolation

A loopful of microorganisms sample was streaked on blood agar base enriched with 7 % defibrinated sheep blood (Oxoid, UK) and MacConkey agar (Oxoid, UK) plates using the quadrant streaking

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method. Both agar plates were incubated aerobically at 37 °C for 24–48 h and examined for characteristic bacterial colonies. Pure culture colonies were selected and sub-cultured on general purpose medium, nutrient agar (Oxoid, UK), and incubated aerobically at 37 °C for 24–48 h for further biochemical identification.

3.12 Antibiotics activity assay

Antibiotic discs were placed on the surface of a Mueller-Hinton agar that has been inoculated with test microorganisms. During incubation, the antibiotics diffuse outward from the discs creating a concentration gradient. After 18-24 hours at a degree of 37 C, the zone diameter of inhibition is measured by means of a ruler and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs (Sockett, 2006),WHO.

3.13 Plant extracts activity assay

3.13.1 Well diffusion method assay

3-13.2 bacteria

According to Obeidat et al. An inoculum suspension was swabbed uniformly to solidified 20 mL Mueller-Hinton Agar (MHA) for bacteria, and the inoculum was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using Glass Pasteur pipettes. Aliquot of 20 µl from each plant(hac & gicek) watery extract (200 mg/ml) was added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion,at the same time control plates done by adding 50 microliters of sterile distilled water in the well instant of plant extract 5 and thereafter incubated at 37 C for 24 h. The resulting inhibition zones were measured in millimeters (mm). Each treatment consists of three replicates and repeated at least twice The rate of the operation was taken

3-13.3 yeasts

The sensitivity test of yeasts to plant extracts using the planning method,loopfull of yeast suspension were planned on Malt Extract Agar filling with different concentrations of the plant extracts in addition to inoculating the control dishes of a container on the medium only or50 microliters of sterile distilled water and then the dishes were incubated at a temperature of 25 ° C for a period Four days later, the result is read on the basis of the intensity of growth

4 - Descution&Results

Today, plants and herbal medicine raw materials constitute a large part of the drugs used in treatment.is to create. The inadequacy of synthetic drugs and therapeutic substances against increasing diseases in recent years and the detection of their side effects have increased the necessity of using natural products.(Kalaycıoğlu and Öner, 1994; Dağcı, 2002. diffusion method was used for qualitative determination of activity and broth micro-dilution was utilized to determine MIC to provide quantitative information. Antimicrobial activities of the plant extracts were determined by agar disc diffusion method by measuring the diameter of the zone inhibition around the discs infused with the plant extracts over the bacterial and fungal culture plates, and the results are presented in (Table 1&2). In our research, the anti-bacterial activity of the methanol extract of the leaves and flowers of the Hedysarum anatolicum plant was shed light on, according to the standard methods used, on eight different types of pathogenic bacteria(Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumonia, Burkholderia cepaciae) and a fungus (Candida albicans). a Table 1 for leaves extract and Table 2 for flower extract. According to Tables 1 and 2, we find that the antibacterial activity of the extract of leaves and flowers was effective against the eight types of bacteria in all concentrations and in varying proportions. But we must point out when comparing the antibacterial activity of leaves and flowers with that of antibiotics, as shown in Table 3, we find that at a concentration of 1:10 the leaves extract had a high antibacterial activity on each of the

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Table:1 Microorganisms Inhibition zon of the Hedysarum anatolicum- leaves extracts

	(IZD --mm)								
	1:10	1:20	1:30	1:40	1:50	1:60	1:70	1:80	control
Staphylococcus aureus	±24	±22	±21	±21	±21	±20	±20	±20	-
E . coli	±20	±25	±24	±23	±21	±21	±21	±20	
Enterobacter cloacae	±28	±15	±15	-	-	-	-	-	-
Streptococcus pyogenes	±29	±28	±27	±25	±20	±15	±15	±15	-
Pseudomonas aeroginosa	±20	-	-	-	-	-	-	-	-
Klebsiella pneumonia	±22	±20	-	-	-	-	-	-	-
Burkholderia cepaciae	±17	±10	±10	±9	±9	-	-	-	-
Candida albicans	±19	±12	-	-	-	-	-	-	-

Table :2 Microorgansms Inhibition zone of the Hedysarum anatolicum –flowers extracts

	(IZD --mm)								
	1:10	1:20	1:30	1:40	1:50	1:60	1:70	1:80	control
Staphylococcus aureus	±28	±25	±24	±23	±22	±21	±21	±20	
E . coli	±16	-	-	-	-	-	-	-	-
Enterobacter cloacae	±17	-	-	-	-	-	-	-	-
Streptococcus pyogenes	±19	±16	±15	±14	±13	±13	±13	±12	-
Pseudomonas aeroginosa	±17	-	-	-	-	-	-	-	-
Klebsiella pneumonia	±24	±23	-	-	-	-	-	-	-
Burkholderia cepaciae	15	-	-	-	-	-	-	-	-
Candida albicans	±22	±19	-	-	-	-	-	-	-

bacteria (Staphylococcus aureus, Streptococcus pyogenes, and Enterobacter cloacae) which were They recorded)IZ) 24mm, 29mm, and 28mm, respectively, while we find in Table 3 that many of the antibiotics did not record an antibacterial effect of (Staphylococcus aureus) except (Refadin)tablets that had an antibacterial effect of 29mm(IZ) . As for the flower extract, we find that it has a high antibacterial activity at a concentration of 1:10 on each of (Staphylococcus aureus, Streptococcus pyogenes, , Enterobacter cloacaeand Klebsiella pneumonia) where(IZ) 29mm, 19mm, 17mm and 24mm were recorded, respectively, and when we compare the results of the two antibiotics in Table 3. We conclude that the antibacterial activity of the flower extract was stronger than that of many antibiotic tablets, and it has similar efficacy to (Refadin tablets for Staphylococcus aureus)&(Ceftaxime tablets for Streptococcus pyogenes).While the antifungal activity of the methanol extract for leaves of (Hedysarum anatolicum) on the fungus (Candida albicans) was 19mm at a concentration of 1:10 and 12mm at a concentration of 1:20, while the antifungal activity of the methanol extract for flowers (Hedysarum anatolicum) was stronger, as we recorded a percentage of inhibiting the fungus (Candida albicans) 22mm(IZ) at concentration of 1:10 and 19mm (IZ)at a concentration of 1:20 . By disseminating these types of studies, antimicrobial activities of plant species were determined and different Identification of antimicrobial substances to be isolated from plant species, in medicine and industry It is important to investigate the possibilities of using it as a pharmaceutical raw material. carries. In antimicrobial activity studies with plant species, the plant content needs to be determined. Further studies are carried out to determine the active substance and to elucidate its chemical structure.

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Table 3		Amst.test/effect of different antibiotics on different microorganisms Antimicrobial sensitivity test							
	Amoxacılıc Clavulanıc acıd	CeftazıdIm	Trımeth0prım sulfa methoxazole	Penicillin	Ampıcıllın	Nitrofuramtion 100mg	Nalidexic Acid	Amikacin	Aztronam
Staphylococcus aureus	---	----	----	----	----	----	-----	-----	----
E . colı	R	R	S 17mm	----	R	S 20mm	R	S 20mm	S 15mm
Enterobacter cloacae	R	R	R	----	R	R 12mm	R	R 12mm	R
Streptococcus pyogenes	----	----	R		R	---	----	---	---
Pseudomonas aerogınosa	----	---	---	R 11mm	---	---	----	-----	R 10mm
Klebsıella pneumonıa	R	R	S 22mm	R	R	R 10mm	R	I 15mm	S 17mm
Burkholderıa cepacıae	-----	R	R	--	---	---	---	---	---

Table 3		Amst.test/effect of different antibiotics on different microorganisms Antimicrobial sensitivity test							
	Amoxacılıc Clavulanıc acıd	CeftazıdIm	Trımeth0prım sulfa methoxazole	Penicillin	Ampıcıllın	Nitrofuramtion 100mg	Nalidexic Acid	Amikacin	Aztronam
Staphylococcus aureus	---	----	----	----	----	----	-----	-----	----
E . colı	R	R	S 17mm	----	R	S 20mm	R	S 20mm	S 15mm
Enterobacter cloacae	R	R	R	----	R	R 12mm	R	R 12mm	R
Streptococcus pyogenes	----	----	R		R	---	----	---	---
Pseudomonas aerogınosa	----	---	---	R 11mm	---	---	----	-----	R 10mm
Klebsıella pneumonıa	R	R	S 22mm	R	R	R 10mm	R	I 15mm	S 17mm
Burkholderıa cepacıae	-----	R	R	--	---	---	---	---	---

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LIST OF ABBREVIATED TERMS

1. MIC\ Minimum Inhibitory Concentration
2. CLSI \ Clinical and Laboratory Standards Institute
3. BHI \ Brain Heart Infusion
4. CFU \ Colony Forming Unit
5. VRSA \ Vancomycin-resistant S. aureus
6. ESBLs \ extended-spectrum β lactamases
7. MRSA \ Methicillin-resistant Staphylococcus aureus
8. WHO:\ World Health Organization
9. MHA \ Mueller hinton aga