

## ***In vitro* Antimicrobial Activity Screening of *Rheum rhabarbarum* Roots**

Kerem Canli<sup>1</sup>, Ali Yetgin<sup>2</sup>, Ilgaz Akata<sup>3</sup>, Ergin Murat Altuner<sup>4</sup>

<sup>1</sup> Dokuz Eylül University, Faculty Of Science, Department Of Biology, Buca, İzmir, TURKEY

<sup>2</sup> Izmir Institute Of Technology, Faculty Of Science, Department Of Molecular Biology, Izmir, TURKEY

<sup>3</sup> Ankara University, Faculty Of Science, Department Of Biology, TR 06100, Ankara, TURKEY

<sup>4</sup> Kastamonu University, Faculty Of Science And Arts, Department Of Biology, Kastamonu, TURKEY

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**Abstract:** *Rheum rhabarbarum*, which is commonly known as rhubarb, has been used as a medicinal herb in different countries. Especially its roots are known to be a traditional medicine in different cultures. Mesir paste was prepared about 500 years ago during Ottoman period as a medicinal paste and *R. rhabarbarum* was one of its ingredients. In this study the *in vitro* antimicrobial activity of ethanol extract of *R. rhabarbarum* roots was investigated against 17 bacterial and 1 fungal strain, namely, *Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386, *Enterobacter aerogenes* ATCC 13048, *Enterococcus durans*, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Listeria innocua*, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescense* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium* SL 1344, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* DSMZ 20044 by using the disk diffusion method. It is observed that ethanol extracts of *R. rhabarbarum* root extracts has antimicrobial activity against all microorganisms tested.

**Keywords-** *Rheum rhabarbarum*, Mesir paste, antimicrobial activity, antimicrobial screening, ethanol extract.

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### **I. INTRODUCTION**

Using plants against diseases is assumed to be as old as human history. Today there is an increasing interest by the scientists to define the secrets of these traditional herbal medicines [1]. The search for new antimicrobial agents has increased mainly because of the increase in infections particularly in developing countries with medically indigent populations and more so because of extensive bacterial resistance to current antimicrobial agents [2]. It is a well-known issue that most of the antimicrobial agents are developed from natural products, which have an antimicrobial potential [3, 4, 5]. Humankind without scientific knowledge discovered new treatment methods through trial and error method in the history [6]. Mesir paste is a traditional special mixture of several herbs and spices used as a medicine, which was founded about 500 years ago during Ottoman period, including *Terminalia citrina* (black chuglam or citrine myrobalan), *Zingiber officinale* (Ginger), *Cuminum cyminum* (Cumin), *Angelica sylvestris* (wild angelica) and *Rheum rhabarbarum* (rhubarb). All ingredients of Mesir paste separately have been used for the treatment of various diseases in Turkish folk medicine for long centuries [7]. In addition to this, especially the synergistic antimicrobial effect thought to be the reason of Mesir paste's healing effect.

World Health Organization (WHO) has predicted increasing antimicrobial resistance as a major threat for the public health for the 21<sup>st</sup> century [8]. In order to prevent spreading of antibiotic resistant infections, scientists have been conducting intensive researches to determine new antimicrobial agents. One way to prevent antibiotic resistance of microorganisms is by using new compounds that are not based on existing antimicrobial agents [9, 10].

In this study the antimicrobial activity screening of *R. rhabarbarum* roots, one of the ingredients of Mesir paste, is investigated against 17 bacterial and 1 fungal strains by using the disk diffusion method.

### **II. MATERIALS AND METHODS**

#### **Extraction Procedure**

All *R. rhabarbarum* samples were ground by a pestle and a mortar. Ethanol (Sigma-Aldrich) was chosen as an extraction solvent in order to extract active substances. Ground samples were shaken in the extraction solvent at 90 rpm for 3 days. The extract was filtered through filter paper (Whatman No. 1) into evaporation flasks. The filtrate was evaporated by a rotary evaporator at 38°C. After evaporation the residues were collected and used to prepare 250 mg.mL<sup>-1</sup> of ethanol extracts.

### Microorganisms

A wide range of microorganisms were selected to test the antimicrobial effect of *R. rhabarbarum*. These strains are *Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386, *Enterobacter aerogenes* ATCC 13048, *Enterococcus durans*, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Listeria innocua*, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescense* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium* SL 1344, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* DSMZ 20044.

The strains were chosen from standard strains as much as possible. Other strains which are not standard were all isolated from food and identified in Ankara University, Faculty of Science, Department of Biology.

### Preparation Of Inocula

All bacterial strains were incubated at 37 °C for 24 hours [11]. But since the requirements for *C. albicans* is different, *C. albicans* was inoculated at 27 °C for 48 hours. Inocula were prepared by transferring morphologically similar colonies of each organism into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland standard having approximately  $10^8$  cfu.mL<sup>-1</sup> for bacteria and  $10^7$  cfu.mL<sup>-1</sup> for *C. albicans* [12-15].

### Disk Diffusion Method

Disk diffusion test was performed as described previously by Andrews [16]. The culture medium was poured into 90 mm sterile Petri dish to give a mean depth of 4.0 mm ± 0.5 mm [17, 18]. 50 µL and 100 µL aliquots of extract was applied on sterile disks of 6 mm diameter end up with 12500 µg and 25000 µg sample on each disk [19, 20]. To get rid of any residual solvent which might interfere with the results, disks were left to dry overnight in sterile conditions [20, 21]. The surfaces of the plates were inoculated using previously prepared inocula containing saline suspension of microorganisms. Inoculated plates were then left to dry for 5-6 minutes at room temperature in aseptic conditions before applying the disks [22]. Disks were firmly applied to the surface of the plate which had an even contact with the agar. Plates were incubated and inhibition zone diameters were expressed in millimetres [23-24].

### Controls

Empty sterile disks and extraction solvent (ethanol) loaded on sterile disks which were dried at sterile conditions to remove solvent as done in the study were used as negative controls. Gentamicin 10 µg used as positive control.

### Statistics

All extracts were tested in triplicate and MACANOVA (version 5.05) was used for statistical analysis of the data. *P* values of < 0.05 were considered statistically significant.

## III. RESULTS AND DISCUSSION

The main aim of this study was to identify the antimicrobial activity of ethanol extracts of *R. rhabarbarum* roots. To do this, disk diffusion test was performed in the study. In this test, extracts were loaded on empty sterile disks and these disks were then applied on a culture medium inoculated with microorganisms. If the extracts were shown activity against these microorganisms, they have caused an inhibition zone. The diameters of the inhibition zones recorded in millimetres are given in Table 1. No activity was observed for the negative controls; extraction solvent and empty sterile disks.

**Table 1.** Disk diffusion test results (Inhibition zones in mm)

	50µL	100µL	Gentamicin
<i>B. subtilis</i> DSMZ 1971	15	17	30
<i>C. albicans</i> DSMZ 1386	18	20	-
<i>E. aerogenes</i> ATCC 13048	11	13	23
<i>E. durans</i>	14	16	14
<i>E. faecalis</i> ATCC 29212	12	16	13
<i>E. faecium</i>	25	28	28
<i>E. coli</i> ATCC 25922	10	14	20
<i>K. pneumoniae</i>	9	10	22
<i>L. innocua</i>	12	13	13
<i>L. monocytogenes</i> ATCC 7644	23	29	28
<i>P. aeruginosa</i> DSMZ 50071	14	17	15

<i>P. fluorescens</i> P1	15	18	12
<i>S. enteritidis</i> ATCC 13075	9	13	24
<i>S. infantis</i>	8	11	24
<i>S. kentucky</i>	8	10	13
<i>S. typhimurium</i> SL 1344	9	11	23
<i>S. aureus</i> ATCC 25923	12	20	24
<i>S. epidermidis</i> DSMZ 20044	12	17	25

“-“: No activity observed.

Results given in Table 1 clearly show that 50  $\mu\text{L}$  ( $12500 \mu\text{g}\cdot\mu\text{L}^{-1}$ ) of *R. rhabarbarum* root samples caused an inhibition zone of 25 mm against *E. faecium*, 23 mm against *L. monocytogenes* ATCC 7644, 18 mm against *C. albicans* DSMZ 1386, 15 mm against *B. subtilis* DSMZ 1971 and *P. fluorescens* P1, 14 mm against *E. durans* and *P. aeruginosa* DSMZ 50071, 12 mm against *E. faecalis* ATCC 29212, *L. innocula*, *S. aureus* ATCC 25923 and *S. epidermidis* DSMZ 20044, 11 mm against *E. aerogenes* ATCC 13048, 10 mm against *E. coli* ATCC 25922, 9 mm against *K. pneumonia*, *S. enteritidis* ATCC 13075 and *S. typhimurium* SL 1344, 8 mm against *S. infantis* and *S. kentucky* where 100  $\mu\text{L}$  ( $25000 \mu\text{g}\cdot\mu\text{L}^{-1}$ ) of *R. rhabarbarum* root samples caused an inhibition zone of 29 mm against *L. monocytogenes* ATCC 7644, 28 mm against *E. faecium*, 20 mm against *C. albicans* DSMZ 1386 and *S. aureus* ATCC 25923, 18 mm against *P. fluorescens* P1, 17 mm against *B. subtilis* DSMZ 1971, *P. aeruginosa* DSMZ 50071 and *S. epidermidis* DSMZ 20044, 16 mm against *E. durans* and *E. faecalis* ATCC 29212, 14 mm against *E. coli* ATCC 25922, 13 mm against *E. aerogenes* ATCC 13048, *L. innocula* and *S. enteritidis* ATCC 13075, 11 mm against *S. infantis* and *S. typhimurium* SL 1344, 10 mm against *K. pneumoniae* and *S. kentucky*.

It is observed that ethanol extracts of *R. rhabarbarum* root extracts has antimicrobial activity against all microorganisms tested.

Conter *et al.* [25] reported that *L. monocytogenes* strains are susceptible to the antibiotics commonly used in human listeriosis treatment, but *L. monocytogenes* is slowly becoming antibiotic resistant and a perpetual surveillance of emerging antimicrobial resistance of this pathogen is critical to ensure effective treatment of human listeriosis. From this point of view, having antibacterial activity against *L. monocytogenes* may very important. Ates and Erdogru [26] identified that ethanol extract of *Juniperus oxycedrus* caused 7 mm of inhibition zone against *L. monocytogenes* whereas *Cinnamomum cassia*, *Glycyrrhiza glabra*, *Coriandrum sativum* and *Pimpinella anisum* observed no activity. On the other hand Gentamicin 10  $\mu\text{g}$  caused 28 mm of inhibition zone against *L. monocytogenes*. In our study we observed 23 mm zone for  $12500 \mu\text{g}\cdot\mu\text{L}^{-1}$  of *R. rhabarbarum* roots and 29 mm zone for  $25000 \mu\text{g}\cdot\mu\text{L}^{-1}$  mg of *R. rhabarbarum* roots. Comparing these results clearly presents that *R. rhabarbarum* roots are highly active against *L. monocytogenes*.

*Enterococcus faecium* has long been thought of as a harmless commensal of the mammalian GI tract. However, *E. faecium* has become an important cause of nosocomial infections. These infections are often difficult to treat owing to the resistance of *E. faecium* to a large number of antibiotics [27]. Mojab *et al.* [28] identified that methanol extract of *Thymus daenensis* caused 8 mm of inhibition zone against *E. faecium* whereas Ilhan *et al.* [29] identified that methanol extract of *Palustriella commutata* observed no activity. In our study we observed 25 mm zone for  $12500 \mu\text{g}\cdot\mu\text{L}^{-1}$  of *R. rhabarbarum* roots and 28 mm zone for  $25000 \mu\text{g}\cdot\mu\text{L}^{-1}$  mg of *R. rhabarbarum* roots, which is equal to Gentamicin 10  $\mu\text{g}$ .

*S. aureus* is known one of the common nosocomial infections in medical intensive care units [30]. Several researchers study antimicrobial activity of some plant extracts on *S. aureus* strains. For example, Nair and Chanda [31] compared 10 medicinal plants antimicrobial effects on *S. aureus* strains, namely *Anethum graveolens*, *Commiphora wightii*, *Emblca officinalis*, *Ficus benghalensis*, *Ficus racemosa*, *Ficus religiosa*, *Ficus tisela*, *Hibiscus cannabinus*, *Mentha arvensis* and *Mimusops elengi*. In this study maximum activity of ethanol extract was shown by *E. officinalis* with 9 mm of inhibition zone. In our study we observed 20 mm zone for  $25000 \mu\text{g}\cdot\mu\text{L}^{-1}$  of *R. rhabarbarum* roots. Comparing these results clearly puts forward how *R. rhabarbarum* roots are active against *S. aureus* when compared to some other higher plants.

#### IV. CONCLUSION

As a result, it can be concluded that there is clear antimicrobial activity of *R. rhabarbarum* roots against all of the strains tested. The results of our study clearly presents that *R. rhabarbarum* roots could have a possible medicinal uses especially against *L. monocytogenes* ATCC 7644, *E. faecium*, *P. fluorescens* P1, *B. subtilis* DSMZ 1971, *S. epidermidis* DSMZ 20044 and *P. aeruginosa* DSMZ 50071.

But further researches are needed to be conducted in order to analyse the active substances and their activity mechanisms in details.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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