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Determination of some biological properties over *Kluyveromyces lactis* 1 of *Rheum ribes* L. (Rhubarb) as a traditional medicinal and food plant

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Abstract
In this study; fatty acid, vitamin, phytosterol, flavonoid and resveratrol contents and antimicrobial activities of *Rheum ribes* L. (rhubarb) extracts treated with *Kluyveromyces lactis* 1 were determined and compared. *R. ribes* is known as both peptic and fibrous worldwide by humans. According to the results obtained; it was observed that total fatty acid, vitamin and phytosterol contents in *R. ribes* (rhubarb) extracts were at certain levels. It was detected that total fatty acid levels; at significant rates, vitamin contents; partly of *R. ribes* extracts prepared with *K. lactis* 1 increased; however, flavonoid contents decreased at different rates. In the study, it was noticed that *R. ribes* had antioxidant and antimicrobial activities at changing rates. When antimicrobial activities of *R. ribes* extracts containing *K. lactis* 1 were analyzed, it was observed that they had effect at changing rates against all of the bacteria, yeasts and dermatophyte fungi except *Staphylococcus aureus* of fatty acid extracts. On the other hand, vitamin and flavonoid extracts demonstrated scarcely any antimicrobial activity. In conclusion, it was detected that *R. ribes*, which has a local plant, had positive effects on the development of *K. lactis* 1 used in this study which is also accepted as a potential probiotic. It was observed that this yeast type developing in extracts obtained from plants affected biological active compounds at changing rates.

Key words: Antimicrobial activity, Fatty acid, Phytosterol, *Rheum ribes*, Vitamin.

1. Introduction

Probiotics are living microorganisms which are taken with nutrients or separately and effects host's health positively by regulating mucosal and systemic immunity, providing microbial balance (Coşkun, 2007). The characteristics of microorganisms to be used as probiotics, such as capabilities of preserving liveliness, acidity, resistance to bile salts, adhesion and antimicrobial activity are defined in various articles (Bozkurt and Aslım, 2004; Önal et al., 2005; Gürsoy et al., 2005; Kültürsay, 2009). It is mentioned in the studies conducted that yeasts should be attached more importance among other microorganisms utilized as probiotics due to their intensively cleaving to intestinal flora and colonization (Mohanty et al., 1996; Gatesoupe, 1999).

Studies on probiotics and recognition of the importance of probiotics led to the development of prebiotics (Aşan and Özcan, 2006). Various peptides, proteins and lipids are prebiotics; however indigestible carbohydrates, fibrous substances are more focused on as prebiotic sources (Anonymous, 2017). As prebiotics are energy sources for beneficial microorganisms, it is assumed that, when goods which include both prebiotics and probiotics are consumed, probiotics will be alive longer and cause additive and even synergistic effect (Coşkun, 2007).

This research study investigates the effect of peptic and fibrous rhubarb plant on the development of *K. lactis* 1 that is accepted as a health-wise useful and potential probiotic microorganism and compares some biological properties. Hence, the importance of the study is emphasized in terms of the positive effect of

vegetable nutrition on probiotics which are useful for the health of living organisms (plant-probiotic relationship).

2. Materials and Methods

R. ribes samples used in this study were obtained from Elazig city in Turkey. Samples were conserved in deep freezer at -20 ºC until they were extracted.

2.1. Extraction of lipids

Wet weight of cell pellets was determined and then they were homogenized with 3/2 (v/v) Hexane-Isopropanol mixture. After the homogenate was centrifuged at 5000 rpm at 4 Cº for 5 minutes, supernatant part was used for fatty acid and ADEK vitamin analysis (Hara and Radin, 1978).

2.2. Preparation of fatty acid methyl esters

A sample of 5 mL was taken from supernatant part and 5 ml of 2% methanolic sulfuric acid was added to it. After it was vortexed, it was left at 50º C for 12 hours and then after it was cooled down to room temperature, 5 mL of 5% sodium chloride (NaCl) solution was added and the mixture was vortexed again. Fatty acid methyl esters were extracted with 5 mL of hexane. After this mixture was treated with 5 mL of 2% $KHCO_3$ solution, hexane phase was evaporated with nitrogen flow and the mixture was analyzed after it was dissolved in 1 mL of hexane. Analysis of fatty acid methyl esters was performed on SHIMADZU GC 17 device (Christie, 1992; Tvrzicka et al., 2002).

2.3. HPLC analysis of ADEK vitamins and sterol amount

Five percent KOH solution was added onto a sample of 5 mL taken from the supernatant part, vortexed and then kept at 85 Cº for 15 hours. Later, the mixture was cooled down to room temperature and was added 5 mL of distilled water and then vortexed. After lipophilic molecules were treated with 2x5 mL hexane, the hexane in the medium was removed. Later, it was dissolved in 1 mL of (1:1, v/v) acetonitrile/methanol mixture and analyzed with Shimadzu brand HPLC device (Katsanidis and Addis, 1999). Chromatograms were recorded at at 320 nm for retinol (vitamin A) and retinol acetate and 215 nm for δ-tocopherol, vitamin D, α-tocopherol, α -tocopherol acetate, 202 nm for phytosterols, 265 nm for vitamin K_1. Identification of the individual vitamins and phytosterols was performed by frequent comparison with authentic external standard mixtures analyzed under the same conditions (Lopez-Cervantes et al., 2006). The results of analyses were expressed as µg/g for each sample.

2.4. DPPH radical scavenging activity

Free radical 25 mg/L DPPH (α,α-Diphenyl-β-picrylhydrazyl) methanolic solution was prepared. During the experiment, plant samples at 25, 50, 100 and 250 µL concentrations were added onto 3.9 mL methanolic solution of DPPH radical, vortexed and then incubated in a dark environment at room temperature for 30 minutes. Absorbance values were read against a blank at 517 nm using a spectrophotometer (Brand-Williams et al., 1995; Hsu et al., 2006). Radical scavenging activity was calculated as %. DPPH radical scavenging activity was calculated by using (%) = [(Controlλ–Sampleλ)/ (Controlλ)] x 100 formula.

2.5. Determination of resveratrol and flavonoid contents

Flavonoid and resveratrol analysis was conducted on HPLC device and all operations were performed at 25 °C (Zu et al., 2006).

2.6. Extraction and analysis of phytosterols

Five percent KOH was added onto the plant sample which was homogenized with hexane/isopropanol alcohol mixture (at 3/2 v/v ratio) and then it was hydrolyzed at 85 ºC. Extraction was treated with n-heptane and analyzed with HPLC device.

2.7. Sugar analysis

Plant sample was homogenized with distilled water. Then, supernatant part was separated from the pellet. After total filtrate volume was determined, it was analyzed with HPLC device and Shim-Pack HRC NH2 (150X4.6 mm, 5µ.) column was used. Acetonitrile + Water (v / v) (%75 / %25) mixture was used as mobile phase (Chromatography A, 2004).

2.8. Test microorganisms

A total of 2 gram+ bacteria (*Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32), 2 gram- bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* FMC 5), 2 yeasts (*Candida albicans* FMC 17, *Candida glabrata* ATCC 66032) and 2 dermatophyte species (*Trichophyton* sp., *Epidermophyton* sp.) were used in the current research. Microorganisms were provided from the Department of Biology, Fırat University, Microbiology Laboratory, Elazığ-Turkey.

2.9. Antimicrobial activity

Antimicrobial tests were carried out by the well agar method using 100 µL of suspension containing 10^6 cells / mL of bacteria, 10^4 cells / mL yeast and cells / mL dermatophyte fungi as per McFarland standard, inoculated into Mueller Hinton Agar (Difco), Malt Extract Agar (Difco) and Sabouroud Dextrose Agar (Oxoid), respectively. Wells were prepared in the plates with the help of cork-borer (0. 85 cm). 10 µL of the flavonoids, vitamins and fatty acids in plants were introduced directly in to the well. Sterilized petri dishes (9 cm diameter) were placed at 4 °C for 2h. Then, the inoculated plates were incubated at 37±0.1°C at 24 h for bacterial strains and also at 25±0.1°C at 72 h for yeast and dermatophyte fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms (Collins and Lyne, 1987; Özçelik, 1992). Wells injected with methanol and hexane served as negative controls. The experimental studies were replicated three times.

2.10. Development of K. lactis 1 and its treatment with R. ribes extract

K. lactis 1 was cultivated in Yeast Malt Extract Broth for its development and reproduction. After absorbance values were read at 517 nm at spectrophotometer, 1% *K. lactis* 1 culture in bouillon (10^4 yeast/ml) was inoculated into prepared minimal well (0.019 M NaCl, 0.022 M KH_2PO_4, 0.049 M Na_2HPO_4, 0.019 M NH_4Cl, 0.002 M $MgSO_4$, 0.011 M Glucose) (Aydın, 1999) with rhubarb extract under sterilized conditions and appropriate pH level (4.8) was maintained. Extracts developed in the minimal well were collected for living cell count after they were read at 6 h., 12 h., 24 h., 36 h., 48 h., 60 h. and 72 h. at 517 nm on the spectrophotometer; then they were cultivated in Malt Extract Agar and left for incubation and colony counts were examined. Samples were centrifuged when development had stopped and pellets were collected. Fatty acid, vitamin, flavonoid and resveratrol levels and antimicrobial activities of these pellets were analyzed. As a control group, same operations were applied on *K. lactis* 1 and rhubarb developed only in minimal well and comparisons were made. The study was performed with 3 parallel experiments.

2.11. Statistical analysis

SPSS 15.0 software was used for statistical analysis of the data. Analysis of variance (ANOVA) and least significant difference (LSD) tests were also used for comparisons of groups and the control group.
The results given as mean ± SEM. $p < 0.001$(very high statistical significance), very low statistical significance, $p < 0.01$ (partially statistical significance), $p < 0.05$ (very low statistical significance) were used in interpreting the differences between the groups.

3. Results

3.1. Sugar contents

When sugar analysis results of plant extract was examined (Table 1), it was observed that all sugar content in the *R. ribes* (rhubarb) extract excluding arabinose was at significant levels.

Table 1. Sugar contents of plant extract.

Sugars	Arabinose	Fructose	Glucose	Saccarose	Maltose
R. ribes	-	0.2207±0.0005	0.1616±0.0005	1.881±0.00050	0.0263±0.005

3.2. Fatty acids and lipide-soluble vitamins and sterol contents

3.2.1.Fatty acids

When fatty acid contents of *R. ribes* (rhubarb) extracts were analyzed (Table 2); it was observed that palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid were present and they contained 16:0 and 18:2 at high levels ($p<0.0001$). It was detected that 16:0, 16:1, 18:1, 18:2, 18:3 levels in the rhubarb extracts treated with *K. lactis* 1 increased and 18:0 levels decreased with compared to control group rhubarb and *K. lactis* 1. The increases in fatty acids levels indicates that *K. lactis* 1*,* which is accepted to be a probiotic, symbiotically exists with rhubarb extract and being affected by the carbon source in the medium, it activates the enzymes responsible for fatty acid synthesis. The decrease in 18:0 fatty acid level indicates that *K. lactis* 1 consumes this fatty acid in rhubarb. Thus it is determined that based on the increase in fatty acid content, this kind of environment is detected to nourish the development of *K. lactis* 1.

Table2. Fatty acid levels of *Rheum ribes* treated with *K. lactis* 1 (µg/g)**.**

Fatty acid	R+KL	R	KL
16:0	268.06±0.58^{cd}	106.30±0.35	246.28±4.32
16:1	200.36±0.33^{cd}	0.00±0.00	20.41±2.20
18:0	86.23±0.68 ^{d}	47.86±0.31	107.23±0.37
18:1	269.23±0.78^{cd}	82.23±0.37	208.06±3.15
18:2	702.04±1.65^{cd}	178.03±0.17	340.13±0.58
18:3	121.60±1.42 ^{d}	62.50±0.35	73.43±0.37
Total µg/g	1395.00±0.00^{cd}	477.10±1.00	999.46±2.47

R+KL: *R. ribes* + *K. lactis* 1, R:*R. ribes*, KL: *K. lactis* 1, cd: $p<0.0001$, d: $p<0.001$

3.2.2. Lipide-soluble vitamins and sterol contents

When rhubarb extracts were analyzed in terms of their vitamin and phytosterol contents (Table 3), it was detected that K_1, K_2, D vitamins δ – tocopherol, α- tocopherol, retinol, retinol acetate, phytosterols; ergosterol, stigmasterol, β-sitosterol were present in the extracts. When compared to the control group rhubarb and *K. lactis* 1, it was detected that in rhubarb extracts treated with *K. lactis* 1*,* K_2, β-sitosterol, stigmasterol, retinol, retinol acetate amounts increased at high levels, while K_1, D vitamins, ergosterol, α-tocopherol and δ – tocopherol amounts decreased. It is thought that the decrease in the level of vitamins is the consumption by the yeast and the increase in the values of other vitamins is based on *K. lactis* 1. Based on these increased results, it is determined that rhubarb has a positive impact on *K. lactis* 1 development.

Table 3. Phytosterol and vitamin levels of *Rheum ribes* (rhubarb) treated with *K. lactis* 1 (µg/g)**.**

Lipophilic vitamins and phytosterols	R +KL	KL	R
Vitamin K_1	0.0073±0.00^{d}	0.17±0.0017	0.0023±0.00048
Vitamin K_2	0.0017±0.00^{d}	0.0005±0.00004	0.0007±0.001
Vitamin D	0.0017±0.00^{d}	0.011±0.0005	0.008±0.0003
α-tocopherol	0.007±0.00^{d}	0.1540±0.012	0.032±0.0071
δ-tocopherol	0.0001±0.00^{cd}	0.0011±0.00006	0.0021±0.001
Retinol	0.0012±0.00^{cd}	0.0002±0.00	0.0003±0.00
Retinol acetate	0.0022±0.0001^{b}	0.0005±0.00	0.0020±0.00
β-sitosterol	0.50±0.0013^{cd}	0.031±0.0015	0.18±0.036
Stigmasterol	0.046±0.00^{c}	0.12±0.00	0.038±0.0083
Ergosterol	0.0054±0.00^{cd}	0.12±0.00	0.026±0.003

R+KL: *R. ribes* + *K. lactis* 1, R:*R. ribes*, KL: *K. lactis* 1, cd: $p<0.0001$, d: $p<0.001$, c:$p<0.01$, b: $p<0.05$

3.3. Flavonoid contents and radical scavenging properties

Acording to flavonoid and resveratrol contents of rhubarb extracts; it was detected that catechin and naringin were not present but, the levels of rutin, Quercetin, naringenin, resveratrol were present at high rates and myricetin, morin and kaempferol at low rates (Table 4). However, it was detected that all present flavonoid compounds decreased in the rhubarb extract treated with *K. lactis* 1 at different levels with respect to the rhubarb (control) ($p<0.0001$, $p<0.001$). In conclusion, decreasing amounts in the rhubarb extract treated with *K. lactis* 1 indicates that that *K. lactis* 1 uses these compounds in rhubarb.

When DPPH (α,α-Diphenyl-β-picrylhydrazyl) free radical scavenging effect of rhubarb was analyzed, it was showed that it has a effective antioxidant activity at 25 and 50 mL concentration (Figure 1).

Table 4. Flavonoid and resveratrol levels of *R. ribes* (rhubarb) treated with *K. lactis* 1 (µg/g) .

Flavonoids	**R +KL**	**R**
Rutin	0.0004 ± 0.00003^{cd}	0.044±0.0077
Myricetin	0.00 ± 0.00001^{cd}	0.001±0.00008
Morin	0.00 ± 0.00^{cd}	0.0002±0.00001
Quercetin	0.0003 ± 0.004^{cd}	0.004±0.00037
Kaempferol	0.0001 ± 0.001^{cd}	0.0008±0.00004
Naringenin	0.00 ± 0.00^{cd}	0.004±0.001
Resveratrol	0.00 ± 0.00^{cd}	0.0011±0.0006

R+KL: *R. ribes* + *K. lactis* 1, R:*R. ribes*, cd: $p<0.0001$

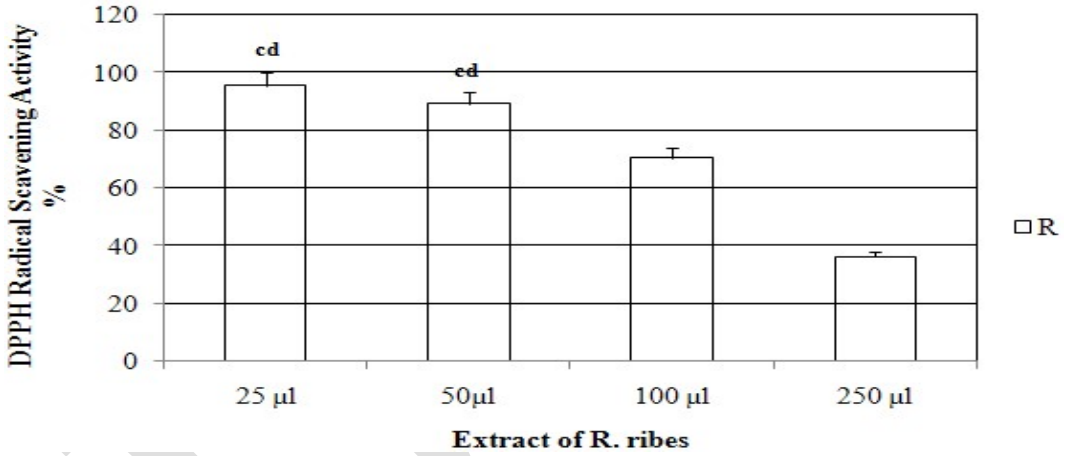

Figure 1. DPPH Radical scavenging activity of *R. ribes* extract.

3.4. ***Antimicrobial activity***

Antibacterial and antifungal effects of fatty acid extracts of plant used in the study are given in Table 6. It was observed that these extracts inhibited developments of bacteria, yeasts and dermatophyte fungi at different rates. According to this, it was detected that it had very low levels of effect against microorganisms such as *E. coli*, *Trichophyton* sp. and (8.5-9.50 mm/inhibition zone) while it had significant antibacterial and antifungal activity over *B. megaterium* (12.5 mm), *C. albicans* (15.5 mm), *C. glabrata* (12.5 mm), *Epidermophyton* sp. (11.5 mm). In additon, it was shown that *R. Ribes* (rhubarb) fatty acid extract did not have any activity on *S. aureus* and *K. pneumoniae*. On the other hand, *R. ribes* fatty acid extracts prepared with *K. lactis* 1 inhibited at changing rates the growth of all bacteria, yeasts and dermatophyte fungi excluding *S. aureus* (8.33-12.33 mm/inhibition zone). Moreover, *K. lactis* 1 fatty acid extracts specifically inhibited the development of yeasts and dermatophyte fungi (11-15 mm) (Table 5, 6) also, they were effective against *S. aureus* (13 mm) and *E. coli* (11 mm).

When the effect of vitamin extracts in *R. ribes* on the development of bacteria, yeasts and dermatophyte fungi was analyzed, it was observed that it had significant antimicrobial activity against *E. coli*; 34.5 mm, *K.*

pneumoniae; 36.5 mm, *C. albicans*; 37.5 mm, *C. glabrata*; 38.5 mm, *Epidermophyton* sp.; 38.5 mm, *Trichophyton* sp.; 38.5 mm, but low levels of antimicrobial activity against *B. megaterium* (18.5 mm). Vitamin extracts containing *K. lactis* 1 prepared from *R. ribes* had very low levels of effect on *S. aureus* and *C. albicans*; however, they did not affect other bacteria, yeast and dermatophyte fungi. On the other hand, *K. lactis* 1 vitamin extracts were effective on all microorganisms except *E. coli*, *K. pneumoniae* (10- 23 mm) (Tablo 5, 6). It is thought that the reason for this reductions is related to consumption by *K. lactis* 1 of these bioactive compounds have antimicrobial activity.

The flavonoid extracts of *R. ribes* were analyzed in terms of their antibacterial and antifungal activities, it was observed that They did not have activity on *E. coli*, *K. pneumoniae* which are bacteria; however, They had high levels of effect on *S. aureus* (10.50 mm), and higher levels of effect on *B. megaterium* (13.50 mm). They did not have any significant effect (Table 6). Also, it was dedected that these flavonoid extracts have antifungal activity on yeast and dermatophyte fungi at different ratios (*C. glabrata*, *Trichophyton* sp.; 8.50 mm, *Epidermophyton* sp.; 9.50 mm and *C. albicans*; 10.50 mm). However, *R. ribes* extracts prepared with *K. lactis* 1 had very low levels of effect on yeasts such as *C. albicans*, *C. glabrata* (8.33-9.33 mm). On the other hand, they did not have any activity on the other all microorganisms (Table 5). Also, it has shown clearly that these data supported results of flavonoid analyses of rhubarb prepared with *K. lactis* 1.

In our study, when antimicrobial activities of *R. ribes* extracts containing *K. lactis* 1 were analyzed, it was observed that they had effect at changing rates against all of the bacteria, yeasts and dermatophyte fungi except *S. aureus* of fatty acid and vitamin extracts. On the other hand, vitamin and flavonoid extracts demonstrated scarcely any antimicrobial activity. It is thought that the reason for this is related to *K. lactis* 1. As previous researchers also indicated, sensitivity of microorganisms against chemotherapeutic materials differs from strain to strain (Kızıl et al., 2004); therefore, some plant extracts may demonstrate at different levels antimicrobial activities. This assertion supports the findings of this study. In addition to, it has become evident that these data presented parallel results with the fatty acid, vitamin and phytosterol, flavonoid and resveratrol analyses conducted in this study.

Table 5. Antimicrobial activities.

Microorganisms	**Inhibition zone (mm)**				
	Antimicrobial activities of fatty acid, vitamin and flavonoid extracts of *R. ribes* (mm) treated with *K. lactis* 1 (mm)			Antimicrobial Activities of Fatty acid,Vitamin Extracts of *K. lactis* 1 (mm)	
	Fatty acid	**Vitamin**	**Flavonoid**	**Fatty acid**	**Vitamin**
E. coli	8.33±0.33	-	-	11	-
K. pneumoniae	10.33±0.33	-	-	-	-
B. megaterium	12.33±0.33	-	-	-	10
S. aureus	-	10.00±0.33	-	13	12
C. albicans	10.33±0.33	9.00±0.33	9.33±0.33[d]	11	14
C. glabrata	8.33±0.33	-	8.33±0.33[b]	13	18
Epidermophyton sp.	10.33±0.33	-	-	17	23
Trichophyton sp.	8.33±0.33	-	-	15	19

Table 6. Antimicrobial activities of fatty acid, vitamin and flavonoid extracts of *R. ribes* (mm).

Microorganisms	Inhibition zone (mm)					
	R. ribes			Control		
	Fatty acid	Vitamin	Flavonoid	Methanol	Hexane	Standart antibiotics
E. coli	8.50±0.50	34.50±0.50	-	-	15.4±0.20	10.3±0.30**
K. pneumoniae	-	36.00±1.00	-	-	14.5±0.30	9.5±0.30**
B. megaterium	12.50±0.50	18.50±0.50	13.50±1.50	-	13.3±0.40	13.4±0.10**
S. aureus	-	36.50±0.50	10.50±0.50	-	12.4±0.10	9.4±0.30**
C. albicans	15.50±0.50	37.50±0.50	10.50±0.50	-	17.2±0.10	18.2±0.20*
C. glabrata	12.50±0.50	38.50±0.50	8.50±0.50	-	11.1±0.20	12.6±0.40*
Epidermophyton sp.	11.50±0.50	38.50±0.50	9.50±0.50	-	9.3±0.30	NT
Trichophyton sp.	9.50±0.50	38.50±0.50	8.50±0.50	-	17.4±0.40	NT

*: Nystatin (Antifungal, 30 µg/disc), **: Streptomycin sulphate (antibacterial,10 µg/disc), Control (methanol and hexzane): 10 µL, NT: not tested

4. Discussion

Today all goods produced as a part of healthy nutrition for a healthy life gain importance all over the world and in this context fibrous goods gain an increasing importance. As various diseases have become widespread such as namely Colon cancer, intestinal obstruction and obesity named as civilization diseases and also diabetics and cardiovascular diseases, forced people to be more conscious about nutrition and as a result of this fibrously rich goods become more appealing (Erbilir, 2006). Also there are approximately 8000 different phytochemicals in the vegetables, fruits and grains. It is difficult to imitate these huge amount of phytochemicals which are balanced in plants (Coşkun, 2005).

No any study was found about fatty acid contents of *R. ribes* (rhubarb). In the studies conducted it is stated that different parts of *R. ribes* are used for ethnomedical purposes (Zargari, 1991; Tabata et al., 1994), also this herb (*Rheum officinale* Baill) has an antiviral effect on herpes virus (Wang et al., 1996) and hypoglycemic effect excluding it has used in treatment of diabetes, hemorhoids, ulcers, diarrhoea (Özbek et al., 2004; Aladdin et al., 2009). However, various phenolic components are detected in 6 types of rhubarb (*Rheum officinale*, *R. palmatum*, *R. tanguticum*, *R. franzenbachii*, *R. hotaoense* and *R. emodi*) *a*nd it is stated that components such as sennosid, antraquinon, stilbene, glucose are present (Ye et al., 2007), chloroform and methanol extracts prepared from roots and bodies rhubarb used for medical purposes, have antioxidant activity and consist total phenolic components such as pyrocatechol, quercetin and flavonoid (Özturk et al., 2007). Chrysophanol, physcion, emodol, anthraquinane and quercetine, 5-deoxyquercetine, quercetine 3-0-galactoside, quercetine 3-0- rutinozit flavonoid are isolated from suckers of *R. ribes*, the only wildering *Rheum* species grown in Turkey (Tosun and Kızılay, 2003). Also, it is stated that *Rheum palmatum* L., *R. tanguticum* Maxium. ex Balf. and *R. officinale* Baill. herbs consist the therapeutical components such as; anthraquinane (e.g. emodin, rhein, chrysophanol, physcion and aloe-emodin and their glycosides), dianthrons (e.g. sennodines A~D), stilbenes (e.g.mrhapontigenin, resveratrol, piceatannol) and tanins (Li et al., 2006).

It is specified that extracts of rhubarb have broad-spectrum antibiotics characteristics and the extracts prepared from the different parts of this herb have antiviral effect (Hudson et al., 2000; Fazly Bazzaz et al., 2005). However, several previous studies have described the traditional knowledge about *R. ribes* and the uses and different needs for them such as medicine, local markets and more (Kaval et al., 2014; Polat et al., 2015; Mükemre et al., 2016; Korkmaz et al., 2016).

Most of the studies conducted with rhubarb support these findings. However, partial differentiations are related to phenolic component levels. The reasons of the level and differentiation of phenolic components include environmental conditions, chemical characteristics of the soil, climate change, collection time, the area where the herb is grown, ripeness process and difference of species. This situation differs based on the region.

Regarded as a potential probiotic yeast; *Kluyveromyces lactis var. lactis* is described as one of the microorganisms in fungal microbiota which is present in kefir grains that are among the nutritious sources of

probiotics (Turan and İlter, 2007). Moreover, it has been detected that used as probiotic; *Kluveromyces lactis* to was intensively adhere property (Kumura et al., 2004; Farnworth, 2005; Aşan Özüsağlam, 2007). In selection and evaluation of *K. lactis*, one of the milk originated yeast strains accepted as potential probiotics, it is determined that *K. lactis* has a high capability of cleaving to caco-2 cells (colon adeno carcinoma cell) which resemble enterocytes (Kumura et al., 2004). For this reason, these yeasts should be given more importance in the studies on probiotics in the future.

There are many studies regarding probiotics and prebiotics, their usage areas, activity, the components they produce, their importance in nutrition, their effects on human health, and reproduction of goods of animal origin and microorganisms used as probiotics, however being an important fibrous herb in nutrition of living beings, *R. ribes* (rhubarb) was not studied for its effects on development of *K. lactis* 1 which is a probiotic yeast and its effect on this yeast. However, two studies are available in this direction that they made in terms of pre and probiotics relationship (Erecevit et al., 2013a; Erecevit et al., 2013b).

This study will be beneficial in observing the effects of probiotic yeasts in foods of plant origin and in terms of herbal nutrition. Moreover; detecting the effects of extracts obtained from probiotic yeasts which are reproduced by vegetable originated nutrients on health-wise harmful pathogen bacteria, yeasts and dermatophyte fungi will contribute the research in this area. In conclusion, under the light of the data obtained, it was detected that *R. ribes* had positive effects on the development of *K. lactis* 1 which is accepted to be a health-wise useful probiotic yeast and *K. lactis* 1 developed inside the extracts obtained from this plant affected active biological compounds at changing rates.

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