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Study on Some Biological Parameters of *Musa spp.* (Banana) are Known as Prebiotic Treated with *Kluyveromyces lactis* 1

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Abstract In this study; fatty acid, vitamin, phytosterol, flavonoid and resveratrol contents and antimicrobial activities of *Musa spp.* (banana) extracts treated with *Kluyveromyces lactis* 1 were determined and compared. *Banana* is known as both prebiotic and fibrous food worldwide by humans.

According to the results obtained; it was observed that total fatty acid, vitamin and phytosterol contents in banana extracts were at certain levels. It was detected that total fatty acid levels; at significant rates, however, vitamin and flavonoid contents decreased at different rates. In the study, it was noticed that Bananahad antioxidant and antimicrobial activities at changing rates. When antimicrobial activities of banana extracts containing *K. lactis* 1 were analyzed, it was observed that they had effect at changing rates against all of the yeasts and dermatophyte fungi except tested bacteria of fatty acid and flavonoid extracts. On the other hand, vitamin extracts demonstrated at different ratio antimicrobial activity.

In conclusion, it was detected that banana, which has a functional food, had positive effects on the development of *K. lactis* 1 used in this study which is also accepted as a effective probiotic.

Keywords *K. lactis* 1, Musa spp. (Banana), Fatty Acids, Phytosterol, Vitamins

Abbreviations

M *Musa spp.;* KL *Kluyveromyces lactis* 1; M+KL *Musa spp. + Kluyveromyces lactis* 1

1. Introduction

Nowadays, the consumption of food products containing probiotics, has increased worldwide due to concerns regarding healthy diet and well being. This trend has received a lot of attention from the food industries, aiming to produce novel probiotic foods, and from researchers, to improve the existing methodologies for probiotic delivery or to develop and investigate new possible applications [1].

In vitro experiments and studies on humans have documented the capacity of some probiotic strains to synthesize vitamin K, folic Acid (vitamin B_9), vitamin B_2, and B_{12}.Therefore, including fermented foods in vegetarian diets will help in overcoming vitamin deficiencies, specially vitamin B_{12} being the limiting micronutrient provided the right microorganisms are used in their fermentation [2]. Although most probiotics are bacteria, some yeasts such as *Saccharomyces* and *Kluyveromyces*, has been found to have effective probiotic properties [3].

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 [4-7].

A prebiotic was first defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” [8]. The products that contain both probiotics and prebiotics are defined as “synbiotics”, and

because the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favors the probiotic compound [9]. Prebiotics are also available naturally the most common in fiber food such as artichoke, celery, leek, asparagus, banana [10].

A focus on the role of gut microbiota to improve health and prevent disease has attracted intense interest in identifying dietary strategies to modulate the gut microbiota. One such dietary strategy includes the intake of prebiotics and dietary fiber, because they can be metabolized by the gut microbiota [1].

Banana is a useful resource as alternative and complementary prebiotics, due to intestinal health, antimicrobial and antioxidant effects. This study investigates the effect of peptic and fibrous banana fruit on the development of *K. lactis* 1 that is accepted as effective probiotic microorganism and compares some biological parameters. Hence, the importance of the study is emphasized in terms of the positive effect of functional foods on probiotics which are useful for the health of living organisms (prebiotic-probiotic relationship). It was observed that this yeast type developing in extracts obtained from fruit affected biological active compounds at changing rates.

2. Material & Methods

Musa spp. (banana) 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 ºC for 5 minutes, supernatant part was used for fatty acid and A, D, E, K vitamin analysis [12].

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 50 ºC 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 [13, 14].

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 ºC 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 [15]. 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 [16]. The results of analyses were expressed as µg/lg for each sample.

2.4. 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.

2.5. 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 [17, 18]. Radical scavenging

activity was calculated as %. DPPH radical scavenging activity was calculated by using (%) = [(Control λ– Sample λ) / (Control λ)] x 100 formula.

2.6. Determination of Resveratrol and flavonoid contents

Flavonoid and resveratrol analysis was conducted on HPLC device and all operations were performed at 25 °C [19].

2.7. 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.

2.8. Sugar analysis

10 gbananasample 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 NH_2 (150 x 4.6 mm, 5µ.) column was used. Acetonitrile + Water (v / v) (%75 / % 25) mixture was used as mobile phase [20].

2.9. Antimicrobial activity

2.9.1. Test Microorganisms

A total of 2 gram +ve bacteria (*Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32), 2 gram -ve 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, Firat University, Microbiology Laboratory, Elazig-Turkey.

2.9.2. 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 [21, 22]. 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 *Musa* spp. (banana) Extract

K. lactis 1was 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 MNa_2HPO_4, 0.019 M NH_4Cl, 0.002 M $MgSO_4$, 0.011 M Glucose) [23] with banana extract under sterilized conditions and appropriate pH level (4.8) was maintained. Extracts developed in the minimal well were collected for living cell countafter 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* 1and banana developed only in minimal well and comparisons were made. The study was performed with 3 parallel experiments.

3. Results

3.1. Sugar Contents

When sugar analysis results of plant extract was examined (Table 1), it was observed that fructose, glucose, saccarose contents in the *Musa* spp. (banana) extract excluding arabinose was at significant levels. However, maltose was at low levels.

Table 1: Sugar contents of banana extract

Sugars	Arabinose	Fructose	Glucose	Saccarose	Maltose
M	0.00±0.00[a]	**0.5812±0.00**	**0.6626±0.00**	**1.2250±0.00500**	0.0025±0.00

M : *Musa spp*.

3.2. Fatty acids and Lipide-Soluble Vitamins and Sterol Contents

Fatty acids

When fatty acid contents of *Musa* spp. (banana) extracts were analyzed (Table 2); it was observed that palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n9), linoleic acid (18:2) and linolenic acid were present and they contained 16:0 and 18:2 at high levels ($p<0.0001$, $p<0.001$). It was detected that 16:1, 18:0, 18:1, 18:2, 18:3 levels in the banana extracts treated with *K. lactis* 1 increased as partial and very little and 16:0 levels decreased with compared to control group banana and *K. lactis* 1 ($p<0.05$, $p<0.01$). The increases in fatty acids levels indicates that *K. lactis* 1, which is accepted to be a probiotic, symbiotically exists with banana 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 banana. 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.

Table 2: Fatty acid levels of *Musa* spp. treated with *K. lactis* 1(µg/1g)

Fatty acid	M+KL	M	KL
16:0	79.06±0.78[c]	100.20±0.10	246.28±4.32
16:1	28.63±0.31[c]	0.00±0.00	20.41±2.20
18:0	32.10±0.26[b]	27.85±0.05	107.23±0.37
18:1	26.36±4.86[b]	21.90±0.10	208.06±3.15
18:2	**99.00±0.50[d]**	61.40±0.10	340.13±0.58
18:3	63.46±1.56[c]	40.75±0.05	73.43±0.37
Total µg/1g	**327.70±4.82[d]**	251.20±1.10	999.46±2.47

16:0: palmitic acid, **16:1:** palmitoleic acid, **18:0**: stearic acid, **18:1:** oleic acid, **18:2** linoleic acid, **18:3:** linolenic acid**, M+KL:** *Musa* spp. +*K. lactis* 1, **M:** *Musa* spp., **KL:** *K. lactis*1, **cd: p<0.0001, d: p<0.001, c: p<0.01, b: p<0.05, a: p>0.05**

Lipide-Soluble Vitamins and Sterol Contents

When banana extracts were analyzed in terms of their vitamin and phytosterol contents (Table 3), it was detected that K_1, D vitamins δ – tocopherol, α- tocopherol, phytosterols; ergosterol, stigmasterol, β-sitosterol were present in the extracts. When compared to the control group banana and *K. lactis* 1, it was detected that in banana extracts treated with *K. lactis* 1 K_1 at high levels, β-sitosterol at partial, retinol, retinol acetate at very little amounts increased while D vitamin, α-tocopherol, ergosterol amounts at high levels and δ – tocopherol, stigmasterol amounts at low levels decreased. It is thought that the decrease in the level of vitamins and sterol 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 banana has a positive impact on *K. lactis* 1 development.

Table 3: Phytosterol and vitamin levels of *Musa* spp. (banana) treated with *K. lactis*1 (µg/1g)

Lipophilic vitamins and phytosterols	M +KL	KL	M
Vitamin K_1	**1.18±0.0033[cd]**	**0.17±0.0017**	0.013±0.00
Vitamin K_2	-	0.0005±0.00004	-
Vitamin D	**0.0002±0.00[cd]**	**0.011±0.0005**	0.006±0.00
α-tocopherol	**0.0023±0.00006[cd]**	**0.1540±0.012**	0.034±0.00
δ-tocopherol	0.0005±0.00[c]	0.0011±0.00006	0.0007±0.00
Retinol	0.0002±0.00[b]	0.0002±0.00	-
Retinol acetate	0.0002±0.00[b]	**0.0005±0.00**	-
β-sitosterol	0.075±0.00003[d]	0.031±0.0015	0.0162±0.00
Stigmasterol	0.027±0.00006[b]	0.12±0.00	0.042±0.00
Ergosterol	0.0002±0.00[cd]	0.12±0.00	0.031±0.001

M+KL: *Musa* spp. + *K. lactis* 1, M: *Musa* spp., KL: *K. lactis*1, **cd:** p<0.0001**, d:** p<0.001**, c:** p<0.01, **b:** p<0.05, **a:** p>0.05

3.3. Flavonoid contents and Radical scavenging properties

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

When DPPH (α,α-Diphenyl-β-picrylhydrazyl) free radical scavenging effect of banana was analyzed, it was showed that it has a effective antioxidant activity at 250 µL concentration (Figure 1).

Table 4: Flavonoid and resveratrol levels of *Musa* spp. (banana) treated with *K. lactis* 1 (µg/1g)

Flavonoids	**M+KL**	**M**
Rutin	**0.00±0.00^{cd}**	0.0003±0.00005^{b}
Myricetin	**0.00±0. 00^{cd}**	0.0001±0.0001^{b}
Quercetin	**0.0001±0.00^{cd}**	0.00±0.00^{a}
Kamferol	**0.00±0. 00^{cd}**	0.0002±0.00001^{b}
Cat	**0.0007±0.0002^{cd}**	0.005±0.00005 ^{d}
Naringin	**0.0009±0.0001^{cd}**	0.00±0.0000 ^{a}
Naringenin	**0.00±0.00^{a}**	0.00±0.00 ^{a}
Resveratrol	**0.00±0.00^{cd}**	0.0010±0.00001^{cd}

M+KL:*Musa* spp. +*K. lactis* 1, **M:***Musa* spp., **cd: p<0.0001, d: p<0.001**

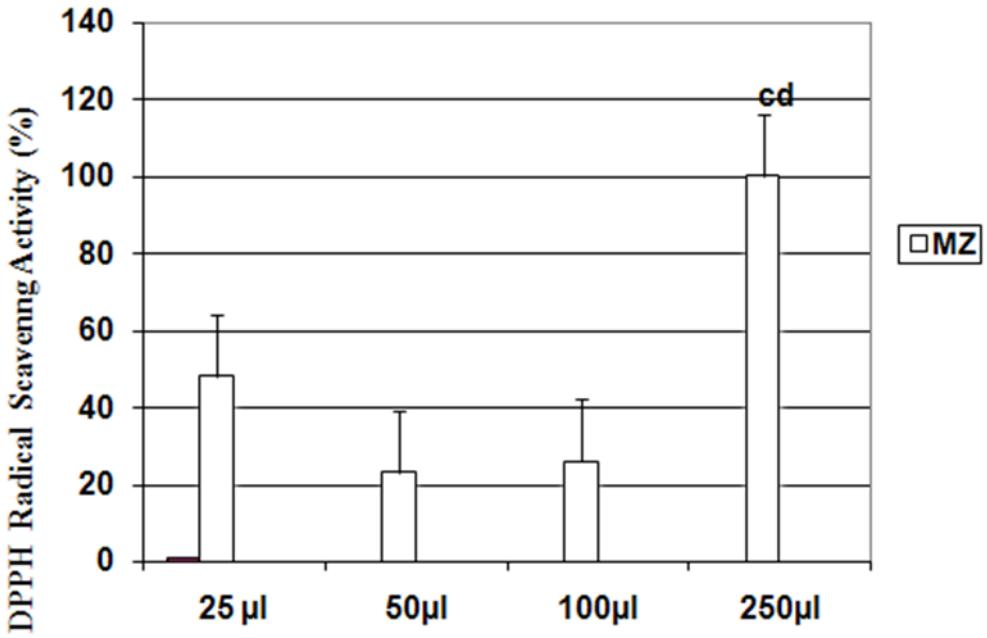

Figure 1: DPPH Radical scavenging activity of Musa spp. extract cd: p<0.0001

3.4. Antimicrobial Activity

Antibacterial and antifungal effects of fatty acid extracts of *Musa* spp. (banana) used in the study are given in Table 5. It was observed that these extracts have antifungal activity on yeast and dermatophyte fungi at higher ratios (13.5-15.5±0.50 mm/inhibition zone)**.** On the other hand, fatty acid extracts were effective at different ratios on all microorganisms except *E. coli* respectively; *B. megaterium* (19.0±1.00 mm), *S. aureus* (8.5±0.50 mm), *K. pneumoniae* (8.5 ±0.50 mm) (Table 6, 7). In addition, it was shown that *banana* fatty acid extracts prepared with *K. lactis* 1 inhibited the development of yeasts and dermatophyte fungi (8-11 mm) (Table 6, 7). However, they did not have activity on *E. coli, K. pneumoniae, S. aureus, B. megaterium* which are bacteria. It

is thought that the reason for these reductions is related to consumption by *K. lactis* 1 of these bioactive compounds have antimicrobial activity.

When the effect of vitamin extracts in banana on the development of bacteria, yeasts and dermatophyte fungi was analyzed, it was observed that it had significant antimicrobial activity against *E. coli*; 14.5±0.50 mm, *K. pneumoniae*; 23.0±1.00mm, *S. aureus;*19.5±0.50 mm, *B. megaterium:* 31.0 mm, *C. albicans*; 23.5±0.50 mm, *C. glabrata*; 27.5±0.50 mm, *Epidermophyton* sp.; 27.0±1.00 mm, *Trichophyton* sp.; 26.5±0.50 mm/inhibition zone. Vitamin extracts containing *K. lactis* 1 prepared from banana had very low levels of effect on *E. coli*, *B. megaterium* (8 mm); however, it had significant levels of effect on *K. pneumoniae* (12 mm), *S. aureus* (10 mm). In addition to, these extracts have antifungal activity on yeast and dermatophyte fungi at higher ratios respectivelly; *C. albicans* (18 mm/inhibition zone), *C. glabrata* (16 mm/inhibition zone), *Epidermophyton* sp. (18 mm/inhibition zone), *Trichophyton* sp. (22 mm/inhibition zone) (Table 6, 7).

Banana flavonoid extracts did not effect on *K. pneumoniae* and *S. aureus*, *C. glabrata*, but they were shown to be effective at different rates despite not being significant in other bacteria, yeast and dermatophyte fungi (8.50±0.50-9.50±0.50 mm/inhibition zone). According to this, it was detected that banana flavonoid extracts prepared with *K. lactis* 1 had very low levels of effect against yeasts and dermatophyte fungi such as *C. albicans*; 8 mm, *C. glabrata*; 8 mm, *Epidermophyton* sp.; 11 mm, *Trichophyton* sp.; 8 mm/inhibition zone) while they did not have antibacterial activity on *E. coli*, *K. pneumoniae*, *S. aureus*, *B. megaterium* (Table 6, 7). Also, it has shown clearly that these data supported results of flavonoid.

In our study, when antimicrobial activities of banana extracts containing *K. lactis* 1 were analyzed, it was observed that they had effect at changing rates against all of the yeasts and dermatophyte fungi except bacteria of fatty acid and flavonoid extracts. On the other hand, vitamin extracts demonstrated at different ratios antimicrobial activity against all of the test microorganisms. It is thought that the reason for this is related to *K. lactis* 1. In addition to, it has become evident that these data presented parallel results with the fatty acid, vitamin and phytosterol, flavonoid, resveratrol analyses conducted in this study.

Table 5: Antimicrobial activities of fatty acid, vitamin and flavonoid extracts of *Musa* spp. (mm)

Microorganisms	**Inhibition zone (mm)**					
	Banana			**Control**		
	Fatty acid	**Vitamin**	**Flavonoid**	**Methanol**	**Hexane**	**Standard antibiotics**
E. coli	-	**14. 5±0.50**	9.50±0.50	-	15.4±0.2	10.3±0.3**
K. pneumoniae	8.50±0.50	**23.0±1.00**	-	-	14.5±0.3	9.5±0.3**
B. megaterium	**19.00±1.00**	**31.0±1.00**	8.50±0.50	-	13.3±0.4	13.4±0.1**
S. aureus	8.50±0.50	**19.5±±0.50**	-	-	12. 4±0.1	9.4±0.3**
C. albicans	**15.50±0.50**	**23.5±±0.50**	9.50±0.50	-	17.2±0.1	18.2±0.2*
C. glabrata	**13.50±0.50**	**27.5±0.50**	-	-	11.1±0.2	12.6±0.4*
Epidermophyton sp.	**15.50±0.50**	**27.0±1.00**	8.50±0.50	-	9.3±0.3	NT
Trichophyton sp.	**13.50±0.50**	**26.5±0.50**	8.50±0.50	-	17.4±0.4	NT

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

Table 6: Antimicrobial activities of fatty acid, vitamin and flavonoid extracts of *Musa* spp. (mm) treated with *K. lactis* 1 (mm)

Microorganisms	**Inhibition zone (mm)**		
	Fatty acid	**Vitamin**	**Flavonoid**
E. coli	-	8	-
K. pneumoniae	-	**12**	-
B. megaterium	-	8	-
S. aureus	-	10	-
C. albicans	11	**18**	8
C. glabrata	9	**16**	8
Epidermophyton sp.	10	**18**	11
Trichophyton sp.	8	8	8

Table 7: Antimicrobial Activities of Fatty acid, Vitamin Extracts of *K. lactis* 1 (mm)

Microorganisms	Fatty Acid	Vitamin
E. coli	11	8
K. pneumoniae	-	**12**
B. megaterium	-	8
S. aureus	**13**	10
C. albicans	11	**18**
C. glabrata	**13**	**16**
Epidermophyton sp.	**17**	**18**
Trichophyton sp.	**15**	**22**

4. Discussion

Functional foods can be defined as foods that are beneficial for health. Although these foods are similar to traditional foods that are beneficial for health as well as basic nutrition and their appearance is consumed on a daily basis [10].

It is determined in a study that Banana fruit has been found to be rich in A and B vitamins [24].

It is determined in a study on the mineral vitamins, ascorbic acid (vitamin C) and mineral content of the banana that banana fruit contains provitamin A and lutein and very high levels of vitamin C (12.7 mg / 100 g), 96.9 µg β-carotene ve 104.9 µg α-carotene/100 g [25]. There is also a study on the biochemical content of banana fruit. With reference to the related study, especially linoleic and ά linolenic acid levels from the fatty acids are highly intense in the banana fruit. These results jibe with our research results [26]. Moreover, it is found in a research about the total lipid content of the banana that the related fruit has a high level of linolenic acid and low levels of linoleic acid [27]. According to the studies conducted, the banana has antifungal and antibacterial effects as well [28].

It is found in a research Green banana flour (BF), produced from Musa Awak, has potential as source of fibre when substituted in noodle products [29].

No any study was found about effects onbananaas probiotic *K. lactis* 1.

5. Conclusions

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 [30]. Fruit, vegetables, cereals and legume products represent promising carriers for probiotic bacteria with good nutraceutical components [31]. For this reason, to these yeasts and fiber foods should be given more importance in the studies on probiotics and prebiotics in terms of symbiotic relationship in the future.

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