

## Nutraceuticals Impact on Probiotics Growth: A Challenge in Synbiotic-Yoghurt Production

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### ABSTRACT

The prebiotic effect of three concentrations of thirteen crude plant extracts: eight aqueous extracts (rosemary, dill, garlic, ginger, flaxseeds, thyme, oat, and moringa leaves); and five Dimethyl sulfoxide (DMSO) extracts (rosemary, dill, garlic, ginger and flaxseeds) on ten different probiotic LAB was studied. Some strains (*Lactobacillus brevis* KP653, *Lactobacillus delbrueckii* subsp. *lactis* KP645, and *Lactobacillus acidophilus* CHA2) were sensitive to the plant extracts, and relative decline in the growth was noticed. While, *Lactobacillus delbrueckii* subsp. *delbrueckii* KT615, *Bifidobacterium longum* 141B, *Lactobacillus plantarum* KP623, *Lactobacillus delbrueckii* subsp. *bulgaricus* YASH2, *Lactobacillus johnsonii* A1, *Lactobacillus casei* YA1, and *Streptococcus thermophilus* T11 were positively influenced with the presence of some extracts. Sixteen synbiotic yoghurt trials were prepared, which produced well-formed set-yoghurt style, similar or close to the control in appearance. Total acceptability scores ranged between 76 and 98%. The highest value was recorded for the control, followed by yoghurt containing rosemary, dill, flaxseeds, thyme, oat, and moringa. While the lowest overall score was recorded for yoghurt containing garlic.

**Keywords:** Nutraceutical, Probiotics, Prebiotics, synbiotic, yoghurt.

### INTRODUCTION

Functional dairy products were recently recognized, as aids reduction of the risk of many diseases (Palmer, 2009; Popkin and Kenan, 2016; Ward, 2016). The dairy industry and market are already the area of most commercial success of functional foods (Kalamian, 2017; Torrence, 2017). Growth of the dairy industry is set to continue with total world milk production of 818 million tons for the year 2016 (Davis and Hahn, 2016). such growth is a good opportunity, since most of the consumers become more aware of the role of nutrition in their diets. However, consumers will not compromise on taste or product quality for healthy products which is an real challenge in functional dairy products development (Tamime and Thomas, 2017). Furthermore, during functional foods designing and manufacturing, bioactive ingredients are added to a food carrier, which can influence acceptance of the overall product (Bimbo *et al.*, 2017).

The term nutraceutical was developed in 1989 by the foundation for innovation in medicine (Kalra, 2003). A nutraceutical is any substance that may be considered as food or part of a food that provides medical or health benefits including prevention and treatment of diseases (DeFelice, 1995). Exopolysaccharides, dietary fibers, polyunsaturated fatty acids, proteins, peptides, amino acids, and antioxidative vitamins are examples of nutraceuticals. Most of the nutraceuticals are well known for their prebiotic activities (El Sohaimy, 2012). Such activities include: Induces selective stimulation of growth and/or activity of intestinal bacteria, potentially associated with health and well-being (Gibson and Roberfroid, 1995; Scott *et al.*, 2014; Mills *et al.*, 2015).

Probiotics are live microorganisms that, when administrated in adequate amounts, confer nutritional and/or therapeutic benefit to the host (Reid *et al.*, 2003). Prebiotic is non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). The ingested prebiotic stimulates the whole indigenous population of probiotic to growth, and the

larger that population, the larger is the number of new bacterial cells. (Roberfroid, 2007).

Synbiotic is a blend of probiotics and prebiotic that beneficially influences the host by enhancing the survival and implantation of probiotics in gastrointestinal tract (Collins and Gibson, 1999).

Dietary antioxidants and polyphenols can act as prebiotics, they are present in plants, herbs, roots and plants leaves and vary considerably from one plant to another. They are considered nutraceuticals which help in combating some of the major health problems such as, cardiovascular diseases, cancer, osteoporosis, arthritis, diabetes, cholesterol etc. Antioxidants and polyphenols play a major protective role by preventing oxidative reactions and working as free radicals scavengers. This has led to a new era, in which the food industry has become a research-oriented sector (Halliwell, 1994; Das *et al.*, 2012). The objective of the present study was to evaluate the effect of some crude plant extracts on the growth of some probiotic strains and the production of synbiotic-yoghurt.

### MATERIALS AND METHODS

#### Strains source

Ten probiotic lactic acid bacteria (LAB) used in this study (*Lactobacillus delbrueckii* subsp. *delbrueckii* KT615, *Lactobacillus brevis* KP653, *Lactobacillus delbrueckii* subsp. *lactis* KP645), were obtained from the Faculty of Agriculture, Saba Bacha, Alexandria University culture collection (FASBAU). Culture of *Bifidobacterium longum* 141B was obtained from Faculty of Agriculture, Shatby, Alexandria University. Cultures of *Lactobacillus plantarum* KP623, *Lactobacillus acidophilus* CHA2, *Lactobacillus delbrueckii* subsp. *bulgaricus* YASH2, *Lactobacillus johnsonii* A1, *Lactobacillus casei* YA1, and *Streptococcus thermophilus* T11 were provided from the Faculty of Agriculture, Al -Basra University, Iraq. These ten strains were considered potentially probiotics since they tolerate bile salts and low pH, comparable to the human digestive system and they show antagonistic activity against some enteropathogenic strains as an indicator.

### Culturing conditions

MRS broth medium (De Man *et al.*, 1960) (Biolife, Italy) was used for reactivating and growing the selected LAB being used in the present study. All strains were maintained for long preservation on MRS slant agar for further study. Bacterial growth from slant culture was reactivated in 10 ml broth medium at 37 °C for 24 h at anaerobic conditions.

### Plants samples

Plants samples mentioned in Table (1) were purchased from markets of Alexandria Governorate, Egypt. All plants samples were collected in clean sterile plastic bags and transported to the lab in ice box. Plants were washed using cold water (4 °C, pH 7), and air-dried at 25 °C for 1 h. Chosen parts of each examined plant were prepared according to the proper methodology.

### Milk

Fresh full fat buffalo's milk was purchased from the local market in Alexandria Governorate. Analysis of milk, was carried out using milk analyzer (3510 Laktostar, Funke Gerber, Berlin, Germany).

### Preparation of crude plant extracts

Aqueous extraction of eight plants (rosemary, dill, garlic, ginger, flaxseeds, thyme, oat, and moringa leaves) were prepared as previously described by Schinella *et al.*, 2002 and Wojdyło *et al.*, (2007). Plant samples (25 g) were ground to a fine paste using a food processor mill (National, Egypt). The samples were extracted with 100 ml distilled water for 1 h using a Magnetic Stirrer (HANNA, Romania). The crude plant extracts were centrifuged at 3500 rpm for 10 min (Electrocenterfuge, EC415, Italy). The extract was then filtered through Whatman paper (Cat. No. 1004, 110 mm). All crude plant extracts sterilized using Millipore filter (0.22 µm micro-filters, GVS, USA) into 15 ml sterile Falcon tubes and then stored at -18 °C until use. DMSO crude extract from rosemary, dill, garlic, ginger and flaxseeds were prepared as described by Romo-Vaquero *et al.* (2014).

Yield of the extracted bioactive compounds, was calculated using the following equation:

$$Y(\mu\text{g} / \text{ml}) = \frac{(X_D - E_D) \times 1000}{V \times X_V}$$

**Where** Y= Yield of crude plant extract (µg/ml); X<sub>D</sub> = Extract density (g/ml); E<sub>D</sub> = density of eluent (g/ml); V= Eluent volume (ml); X<sub>V</sub> = Extract volume (ml)

### Determination of antioxidant capacity (AOC) and total phenolic compounds (TPC) in crude plant extracts

The DPPH assay (Moo-Huchin *et al.*, 2014) was employed to estimate the AOC, where inhibition (%) of DPPH was calculated according to the following equation:

$$\frac{A_0 - A_1}{A_0} \times 100$$

**Where** A<sub>0</sub> is the control absorbance, A<sub>1</sub> is the sample absorbance measured at 517 nm.

TPC of ethanol extracts were prepared as previously described by González-Aguilar *et al.* (2007) with minor modifications. Total phenol and flavonoid

compounds were extracted from 10 g of sample previously homogenized in a 15ml of ethanol (80%) and distilled water. The homogenate was incubated at 60 °C for 60 min and filtered using SELECTA, Nr. 595, 11 cm, (Germany). The volume of pooled solution was brought up to 50 ml. Concentrations of total phenols was measured as described by Singleton and Rossi (1965) with some modifications. Extracts (50 µl) were mixed with 3ml of H<sub>2</sub>O, 250 µl of Folin and Ciocalteus phenol reagent 1 N. After 8 min of equilibrium time, 750 µl of Na<sub>2</sub>CO<sub>3</sub> (20%) and 950 µl of H<sub>2</sub>O were added to the extracts, previous incubated at room temperature for 120 min. The absorbance was measured at 760 nm with an UV-Vis Spectrophotometer (Cary, model 50 Bio, Varian, Italy).

TPC in all samples was determined as described by Khalil and Frank (2010). The amount of TPC was expressed as milligram(s) of Gallic acid equivalents (GAE) per gram of initial weight (IW) according to the following equation (Francisco and Resurreccion, 2009).

$$GAE \frac{\text{mg}}{\text{IW}(\text{g})} = \frac{(A_{760} - b)}{m} \times \frac{\text{yedm}(\text{mg})}{\text{IW}(\text{g})} \times \frac{1\text{mg}}{1000\mu\text{g}}$$

**Where** A is the absorbance at 760 nm, b is the y-intercept of the standard curve, and m is slope of standard curve and yedm is yield of the extracted dry matter.

### Biomass production development

Strains inoculums (200 µl), from active subcultures, were added to 9 ml MRS broth medium. Where, plant extracts were added at levels of 200 µl, 400 µl, and 600 µl in addition to 600 µl, 400 µl and 200 µl distilled water, respectively, to reach a final volume of 10 ml. Bacterial growth was monitored by measuring the optical density at 600 nm (OD<sub>600</sub>) using a Spectrophotometer (APEI PD 303, Japan) for 24h. Afterwards, a growth curve was created for each trial.

### Data analysis

The average change rate in OD readings were calculated across the growth curve and the values were expressed as Δ OD at 600 nm in Parito chart.

### Synbiotic-yoghurt production

Synbiotic yoghurt was prepared using pasteurized (5s/ 80°C) buffalo milk and yoghurt starter bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*). Starter culture was added at 42 °C and mixed. In addition, one probiotic culture (*Bifidobacterium longum* 141B, 0.33% (w/v) ~10<sup>7</sup> cfu/ml) was separately added. Afterwards, 1.5% or 2.0% (w/v) of eight prebiotics were added to make sixteen different combinations. The inoculated milk was poured in cups, incubated at 42 °C for 3 h until the pH 4.6 reached, and was followed by rapid cooling.

### pH determination

pH values of the crude extracts, milk, and yogurt samples were measured using a pH meter (Jenway 3505, England)

### Sensory evaluation

This was carried out by ten panelists. Flavour, appearance, and texture of yoghurt samples were evaluated. A predetermined list of 21 sensory attributes

was used to describe the sensory characteristics of yogurts (Stone *et al.*, 2012).

## RESULTS AND DISCUSSION

### Crude Plants extracts

Yield of aqueous extracts of rosemary, dill, garlic, ginger, flaxseeds, thyme, oat, and moringa leaves and five DMSO extracts of rosemary, dill, garlic, ginger and flaxseeds is shown in Table (1). It was noticed that, when DMSO was used, either 10% or 99%, more yield was achieved in contrast with aqueous extract of the same plant. DMSO did not affect the bacterial growth. Similar observation was proofed by Wadhvani *et al.* (2009); Gomaa (2010).

Data also revealed that garlic gave the highest yield when DMSO of 99% was used as an eluent (72.0 µg/ml). While water extract of ginger gave the lowest yield (1.0 µg/ml). Concerning water extraction, the

highest concentration was observed in case of oat (6.7 µg/ml), followed by rosemary, dill, moringa, thyme, and flaxseed, since the values were 5.7, 5.5, 3.3, 1.3, and 1.2 µg/ml, respectively. Extracts yield of rosemary, dill, and flaxseeds using DMSO 10% were 54.0, 29.0, and 13.7 µg/ml, correspondingly. Ginger showed more than twelve folds the yield when DMSO 99% was used as an eluent (12.8 µg/ml), compared with water extraction. These findings are in agreement with Zhang *et al.* (2014).

Some review articles compared the extractability of the prebiotic arabinoxylans from cereal using different techniques. It was concluded that the yield achieved by water extraction was always lower than that achieved by chemical and enzyme methods (Zhang *et al.*, 2014; Mensink *et al.*, 2015; Ruthes *et al.*, 2015). Therefore, using solvents such as DMSO is suggested as a mean to increase prebiotic yield from different plants.

**Table 1. Yield of bioactive compounds in crude plants extracts.**

Common name	Plant Species	source	Eluent		
			Distilled Water	DMSO 10%	DMSO 99%
Rosemary	<i>Rosmarinus officinalis</i>	Leaves	5.7 µg/ml	54.0 µg/ml	N.D
Dill	<i>Anethum graveolens</i>	Leaves	5.5 µg/ml	29.0 µg/ml	N.D
Garlic	<i>Allium sativum</i>	Cloves	1.25 µg/ml	N.D	72.0 µg/ml
Ginger	<i>Zingiber officinale</i>	Rhizome	1.0 µg/ml	N.D	12.8 µg/ml
Flaxseeds	<i>Linum usitatissimum</i>	Seeds	1.2 µg/ml	13.7 µg/ml	N.D
Thyme	<i>Thymus vulgaris</i>	Leaves	1.3 µg/ml	N.D	N.D
Oat	<i>Avena sativa</i>	Seeds	6.7 µg/ml	N.D	N.D
Moringa	<i>Moringa oleifera</i>	Leaves	3.3 µg/ml	N.D	N.D

N.D = not determined; DMSO = Dimethyl sulfoxide

### Total phenolic compounds (TPC)

Average of the TPC in ethanolic extracts of all plants extracts ranged from 10.70 to 205.23 mg GAE/g (Table 2). The highest TPC value among all plant extracts was observed in moringa leaves, whereas the lowest TPC in oat. The other six plants (ginger, thyme, rosemary, garlic, flaxseeds and dill) recorded values of 123.56, 112.61, 104.99, 61.89, 57.85, and 49.28 mg GAE/g, respectively, in water extracts. In contrast, plants extracts using DMSO showed lower TPC than aqueous extracts (Table 2). Overall, TPC data provide convincing evidence that all trials have high TPC levels detected in ethanolic extracts. These results are in agreement with the previous work (Khalil and Gomaa, 2016).

### Antioxidants capacity (AOC)

Data in Table 2 show that the highest AOC value among all extracts was observed in rosemary, dill, and moringa (99% inhibition). The lowest value was observed in oat (56% inhibition). Ginger, garlic, and flaxseeds recorded values ranged between 71 and 98% inhibition. Generally, DMSO extracts showed higher proportion of antioxidants than aqueous extracts.

All trials in this study contained TPC and AOC among other preferred characteristics, which make most of them very promising when added to any dairy product with positive impact on human health. In general, high consumption of vegetables, fruits and herbs with their antioxidants has been associated with a lowered incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis and

immune system decline etc. (Langseth, 1995; Leong and Shui, 2002).

**Table 2. Total phenolic compounds (TPC), antioxidants capacity (AOC), and pH of water and DMSO extracts of eight plants.**

Plant	Solvent	TPC	AOC	pH
		(mg GAE/g extract)	(DPPH % inhibition)	
Rosemary	Water	104.99	79	4.18
	10% DMSO	46.89	99	6.96
Dill	Water	49.28	83	4.36
	10% DMSO	37.37	99	6.13
Ginger	Water	123.56	80	6.02
	99% DMSO	51.66	98	ND
Garlic	Water	61.89	90	5.80
	99% DMSO	18.56	94	ND
Flaxseeds	Water	57.85	71	4.85
	10% DMSO	45.94	90	5.75
Thyme	Water	112.61	81	3.45
Oat	Water	10.70	56	3.39
Moringa	Water	205.23	99	4.18

### pH

pH values of all samples ranged between 3.39 and 6.96 as shown in Table (2). The lowest pH among all treatments was of 3.39 in water extract of oat, followed by the water extracts of thyme, rosemary, moringa, dill, garlic, and ginger they recorded 3.45, 4.18, 4.18, 4.36, 5.80, 6.02 pH values respectively. Concerning 10% DMSO extracts, the highest value was recorded in case of rosemary (6.96), while flaxseeds was of the lowest (5.75) and dill recorded 6.13. When 99% DMSO was used as solvent pH was not determined.

### Biomass production development

Biomass production development was studied for the ten strains, where plant extracts were added in three concentrations (200  $\mu$ l, 400  $\mu$ l, and 600  $\mu$ l) in 10 ml MRS broth medium. Bacterial growth was monitored and the biomass production data were analysed. The average change rate in OD readings were calculated, values were expressed as  $\Delta$  OD<sub>600</sub> nm.

#### *Lactobacillus delbrueckii* subsp. *delbrueckii* KT615

Figure (1) shows the effect of different plant extracts on the growth of *Lactobacillus delbrueckii* subsp. *delbrueckii* KT615 probiotic strain during 24 h of incubation. Limited growth was noted, since the highest recorded  $\Delta$  OD<sub>600</sub> was 0.115 for control. Thyme, moringa, garlic, rosemary, dill, and oat aqueous extracts and ginger DMSO extract showed stimulative effect in this respect.

Concerning garlic, dill, rosemary, and flaxseeds DMSO extracts, they showed  $\Delta$  OD<sub>600</sub> less than the control. All DMSO extracts suppressed the bacterial growth as compared with the control. This is not due to the presence of DMSO itself since it did not affect the bacterial growth (Wadhvani *et al.*, 2009) and ginger DMSO extract gave  $\Delta$  OD<sub>600</sub> value higher than the control.

#### *Lactobacillus brevis* KP653

Data in Figure (1) show that the probiotic strain *Lactobacillus brevis* KP653 grew well in the broth media containing different plant extracts, except two concentrations of moringa aqueous extract (200 and 400  $\mu$ l), since the control recorded 0.316  $\Delta$  OD<sub>600</sub>. As regards to moringa, the highest  $\Delta$  OD<sub>600</sub> value was 0.343 unit for the concentration of 400  $\mu$ l, followed by the lower concentration (200  $\mu$ l) which recorded very close value to the control (0.319 unit). In contrast the highest concentration (600  $\mu$ l) showed  $\Delta$  OD<sub>600</sub> value lower than the control (0.271).

#### *Lactobacillus delbrueckii* subsp. *lactis* KP645

Although all plant extracts negatively affected the growth of probiotic strain *Lactobacillus delbrueckii* subsp. *lactis* KP645, compared to the control, the bacterial growth in most extracts was relatively high. All trials showed  $\Delta$  OD<sub>600</sub> lower than control, with range of 0.304 and 0.045 (Figure 1).

#### *Bifidobacterium longum* 141B

Probiotic culture of *Bifidobacterium longum* 141B grown in the presence of different plants extracts showed limited growth, since the  $\Delta$  OD<sub>600</sub> of control was 0.144 unit (Figure 2). The data also indicate that most plant extracts, enhanced the bacterial growth. Generally low variation was observed between treatments, since all  $\Delta$  OD<sub>600</sub> values ranged between 0.214 to 0.103. Relatively high  $\Delta$  OD<sub>600</sub> was observed in case of rosemary and moringa aqueous extracts (200  $\mu$ l) as compared to the control.

#### *Lactobacillus plantarum* KP623

Data in Figure (2) indicate that, this strain performed in all trials similarly, or even better than the control.  $\Delta$  OD<sub>600</sub> values ranged between 0.443 and 0.169. Better growth was found in case of thyme, oat, and moringa aqueous extracts at all concentrations (200, 400, and 600  $\mu$ l), compared to the control, except for oat

aqueous extracts at 600  $\mu$ l, which nearly matched the control. The lowest  $\Delta$  OD<sub>600</sub> was detected in case of garlic DMSO extract at all three concentrations.

#### *Lactobacillus acidophilus* CHA2

The growth performance of the probiotic strain *Lactobacillus acidophilus* CHA2 was affected by different plants extracts, where the growth slightly decreased, compared to the control (0.313  $\Delta$  OD<sub>600</sub>). Its growth ranged between 0.307 and 0.112  $\Delta$  OD<sub>600</sub> as shown in Figure (2).

#### *Lactobacillus delbrueckii* subsp. *bulgaricus* YASH2

Results (Figure 3) revealed that, bacterial growth of this strain in all trials was affected with various degrees,  $\Delta$  OD<sub>600</sub> values ranged between 0.665 and 0.177. The control achieved 0.452  $\Delta$  OD<sub>600</sub> as average. Higher  $\Delta$  OD<sub>600</sub> values were observed in case of thyme, oat, moringa and rosemary aqueous extracts at all concentrations (200, 400, and 600  $\mu$ l) except for rosemary aqueous extracts at 600  $\mu$ l which recorded  $\Delta$  OD<sub>600</sub> values of 0.376 unit.

#### *Lactobacillus johnsonii* A1

Probiotic culture of *Lactobacillus johnsonii* A1 showed very good growth, whereas the control achieved 0.452  $\Delta$  OD<sub>600</sub> as average (Figure 3). Generally, the culture maintained viability and good growth at all trials and concentrations.  $\Delta$  OD<sub>600</sub> values ranged between 0.459 and 0.122. It exhibited better growth in trials containing thyme aqueous extracts at all concentrations (200, 400, and 600  $\mu$ l), moringa, oat, and rosemary aqueous extracts at only two concentrations 200, and 400  $\mu$ l and flaxseeds aqueous extracts at 200  $\mu$ l as compared to control.

#### *Lactobacillus casei* YA1

Figure (3) shows the growth performance of the probiotic strain of *Lactobacillus casei* YA1. Very good growth could be detected in all treatments,  $\Delta$  OD<sub>600</sub> ranged between 0.391 and 0.163. The control recorded 0.344  $\Delta$  OD<sub>600</sub>. Moringa, oat, and thyme aqueous extracts at concentrations 200, and 400  $\mu$ l showed higher  $\Delta$  OD<sub>600</sub> values than control. No trial suppressed the growth of *Lactobacillus casei* YA1. However, the lowest  $\Delta$  OD<sub>600</sub> value among all trials recorded in case of the highest concentration of flaxseeds DMSO extract.

#### *Streptococcus thermophilus* T11

Probiotic culture of *Streptococcus thermophilus* T11 grew well in all treatments,  $\Delta$  OD<sub>600</sub> ranged between 0.513 and 0.197. The control recorded 0.431  $\Delta$  OD<sub>600</sub> in average. Moringa and oat at all concentrations and thyme at 400  $\mu$ l aqueous extracts enhanced the growth where  $\Delta$  OD<sub>600</sub> values were higher than the control. The lowest  $\Delta$  OD<sub>600</sub> value among all trials recorded in case of the highest concentration of garlic DMSO extract (Figure 3).

Generally, all data proved that, not any extract suites every strain, each strain was affected differently than the others. Some strains were sensitive to the presence of plant extracts in the medium (*Lactobacillus brevis* KP653, *Lactobacillus delbrueckii* subsp. *Lactis* KP645, and *Lactobacillus acidophilus* CHA2), which led to relative decline in growth, while others were positively influenced in the presence of some extracts. These results can be used to design new synbiotics functional dairy products.

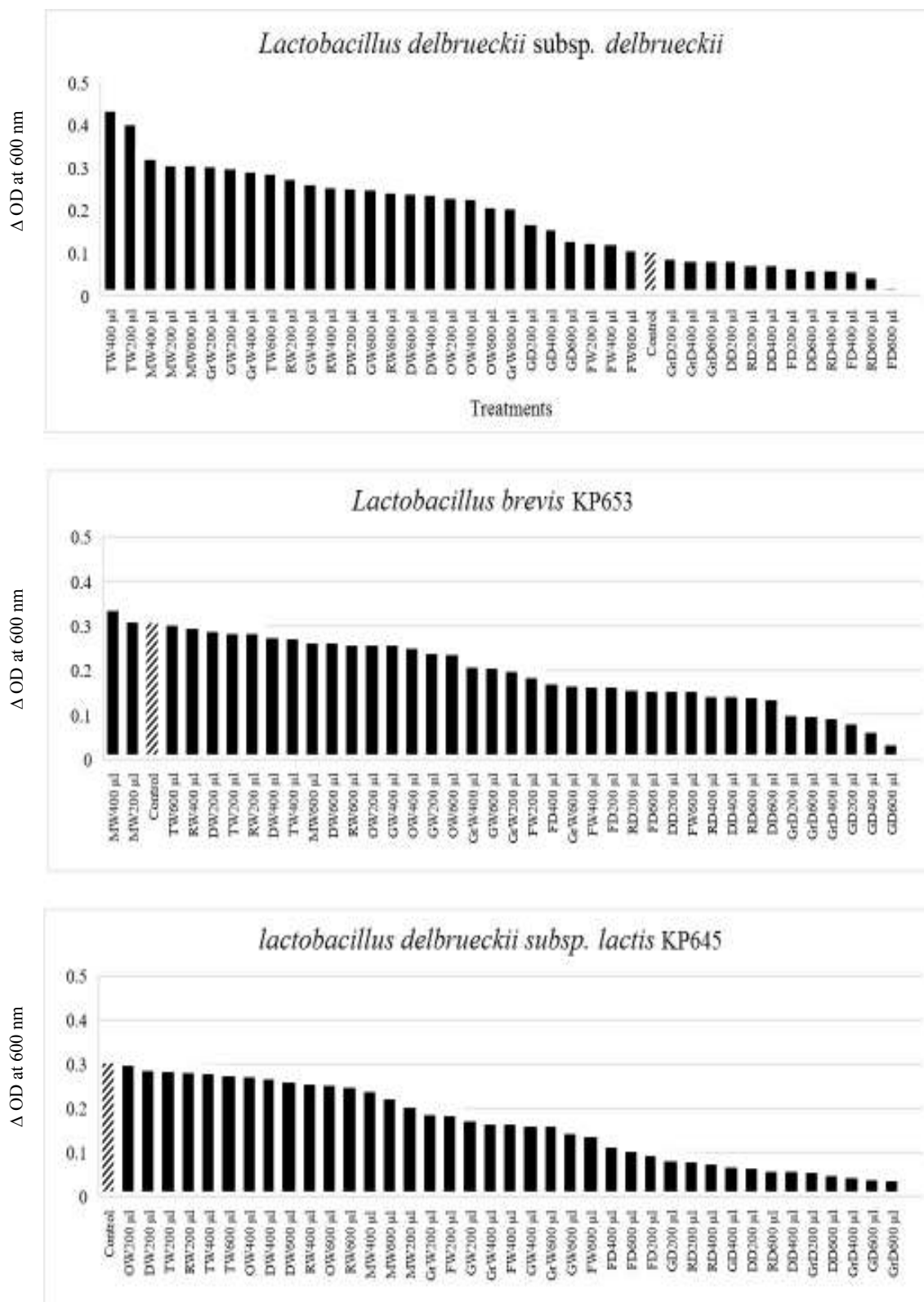


Figure 1. Bar chart where the average change rate in OD at 600 nm ( $\Delta OD_{600}$ ), during 24 h at 37 °C, were represented in descending order for eight aqueous extracts: rosemary (RW), dill (DW), garlic (GrW), ginger (GW), flaxseeds (FW), oat (OW), thyme (TW) and moringa leaves (MW); and five DMSO extracts: dill (DD), garlic (GrD), ginger (GD) and flaxseeds (FD) at three concentration each (200, 400, and 600  $\mu$ l/10 ml medium) in addition to control.

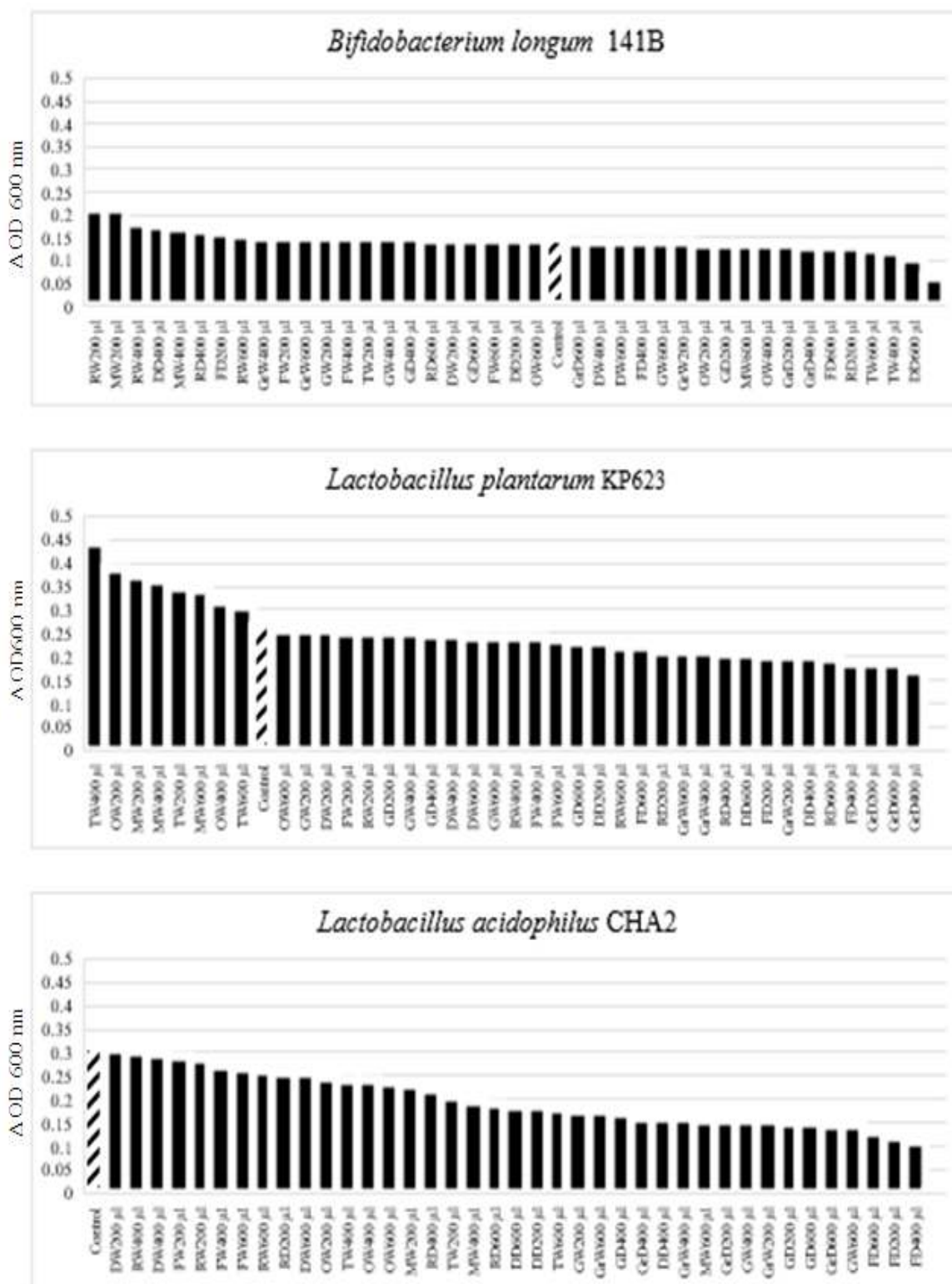


Figure 2. Parito chart where the average change rate in OD at 600 nm ( $\Delta OD_{600}$ ), during 24 h at 37 °C, were represented in descending order for eight aqueous extracts: rosemary (RW), dill (DW), garlic (GrW), ginger (GW), flaxseeds (FW), oat (OW), thyme (TW) and moringa leaves (MW); and five DMSO extracts: dill (DD), garlic (GrD), ginger (GD) and flaxseeds (FD) at three concentration each (200, 400, and 600  $\mu$ l/10 ml medium) in addition to control

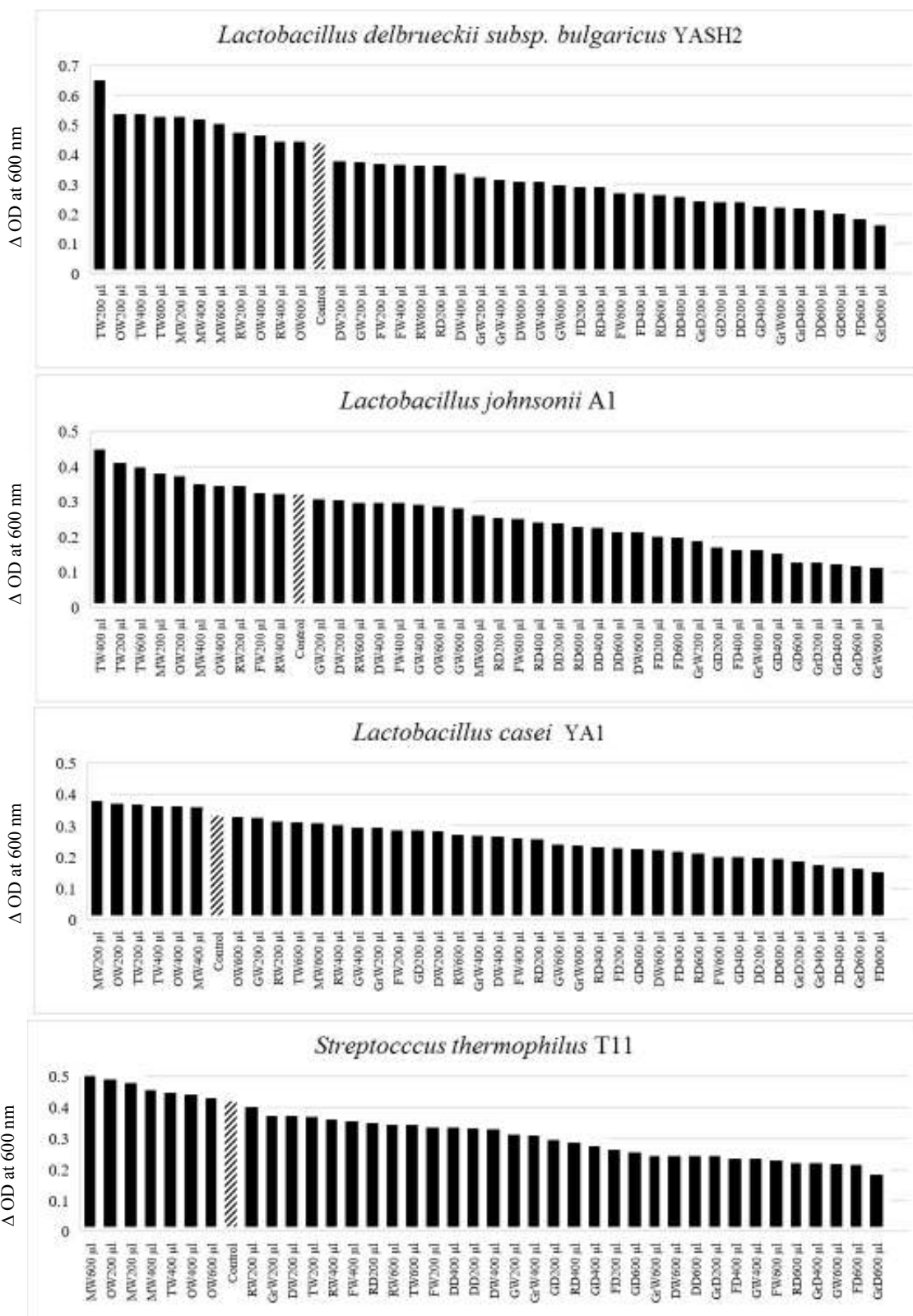


Figure 3. Bar chart where the average change rate in OD at 600 nm ( $\Delta OD_{600}$ ), during 24 h at 37 °C, were represented in descending order for eight aqueous extracts: rosemary (RW), dill (DW), garlic (GrW), ginger (GW), flaxseeds (FW), oat (OW), thyme (TW) and moringa leaves (MW); and five DMSO extracts: dill (DD), garlic (GrD), ginger (GD) and flaxseeds (FD) at three concentration each (200, 400, and 600  $\mu$ l/10 ml medium) in addition to control.

**Sensory evaluation of the prepared synbiotic yoghurt**

Table (3) demonstrates the scores for sensory attributes of yoghurt samples. Evaluating the flavour scores of samples showed that samples containing oat

gained the highest scores that was close to the control. Samples containing rosemary, ginger, flaxseeds, and thyme achieved higher scores than the samples containing dill, garlic and moringa

**Table 3. Sensory evaluation of synbiotic yoghurt made with two concentrations of eight plants aqueous extracts**

Aqueous extracts	concentration (%)	Flavour (smell and taste)		Appearance		Texture		Overall score 100%
		Score <sup>a</sup>	Description	Score <sup>a</sup>	Description	Score <sup>a</sup>	Description	
Control	0.0	5	Creamy, Sweet	5	Thick	5	Homogeneous, Light	98
Rosemary	1.5	4	Creamy, Sweet	4	Thick	4	Homogeneous, Light	95
	2.0	4	Creamy, Sweet	4	Thick	4	Homogeneous, Light	94
Dill	1.5	3	Creamy, Sweet	4	Thick	5	Homogeneous, Light	93
	2.0	3	Creamy, Sweet	4	Thick	3	Homogeneous, Light	95
Garlic	1.5	3	Creamy, Sweet	4	Thick	3	Homogeneous, Light	79
	2.0	3	Creamy, Sweet	4	Thick	3	Homogeneous, Light	76
Ginger	1.5	4	Creamy, Sweet	4	Thick	4	Homogeneous, Light	90
	2.0	4	Creamy, Sweet	4	Thick	4	Homogeneous, Light	92
Flaxseeds	1.5	4	Creamy, Sweet	5	Thick	4	Homogeneous, Light	93
	2.0	4	Creamy, Sweet	5	Thick	4	Homogeneous, Light	91
Thyme	1.5	4	Creamy, Sweet	4	Thick	4	Homogeneous, Light	90
	2.0	4	Creamy, Sweet	4	Thick	4	Homogeneous, Light	94
Oat	1.5	5	Creamy, Acidic	5	Thick	3	Homogeneous, Light	88
	2.0	4	Creamy, Acidic	5	Thick	3	Homogeneous, Light	86
Moringa	1.5	3	Creamy, Acidic	5	Thick	4	Homogeneous, Light	95
	2.0	3	Creamy, Acidic	4	Thick	4	Homogeneous, Light	96

Score<sup>a</sup>: 1=bad; 2=fair; 3=good; 4=very good; 5=excellent

All sixteen-trials produced well-formed set-yoghurt style, similar or close to the control in appearance. Appearance scores of yoghurt samples containing oat, flaxseeds in both concentrations (1.5 and 2.0%) and moringa in its lower concentration (1.5%) were similar to the control. Slightly lower score (4) was given in case of rosemary, dill, ginger, and garlic in addition to moringa in its higher concentration (2.0%).

Comparing texture scores of samples revealed that the highest score was recorded for the sample containing 1.5% dill aqueous extract, while the sample containing rosemary, ginger, flaxseeds, thyme, and moringa ranked lower in score than the control. Texture scores of yoghurt samples containing garlic, oat, and dill 2.0% were less.

Total acceptability scores of all samples ranged between 76 and 98%. The highest value was recorded for control, while yoghurt samples containing rosemary, dill, flaxseeds, thyme, and oat aqueous extracts showed excellent overall score (>90%). However, moringa gave very good overall score (85 and 86%) for trials containing 1.5 and 2.0% aqueous extract respectively. The lowest overall score was recorded for trials containing garlic (79 and 76%) for trials containing 1.5 and 2.0% aqueous extract respectively. It should be mentioned that the scores of all samples were higher than unacceptable limit (60%).

Acetaldehyde, the main factor of yoghurt flavour, which is majorly made from conversion of threonine to acetaldehyde catalyzed by threonine aldolase of *L. delbrueckii* subsp. *bulgaricus*, is converted to ethanol by probiotics that produce alcohol dehydrogenase. Therefore, probiotic yoghurts do not have the typical yoghurt flavour (Ranathunga and Rathnayaka, 2013). This could be the reason for the above results. However, it is clear that, all plant extracts used in this study were acceptable and can be used in dairy products.

**CONCLUSION**

Concerning the extraction process, the data revealed that garlic gave the highest yield when DMSO 99% was used as an eluent (72.0 µg/ml). While water extract of ginger gave the lowest yield (1.0 µg/ml). TPC

data showed that, all trials had high TPC levels that detected in ethanolic extracts. AOC value of all extracts ranged between 56 and 99% inhibition. The lowest value was observed in oat. Generally, DMSO extracts showed higher proportion of antioxidants than aqueous extracts. Some strains were sensitive to the presence of plant extracts (*Lactobacillus brevis* KP653, *Lactobacillus delbrueckii* subsp. *lactis* KP645, and *Lactobacillus acidophilus* CHA2), which led to a relative decline in growth, while others were activated in the presence of some extracts in comparison with the control. The results also indicated that the yoghurt may be a good carrier for developing synbiotic yoghurt. Further research should be carried out in this respect.

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### تأثير المغذيات العلاجية على نمو بكتريا بروبيوتك: تحدي في انتاج اليوجورت السينيوتك

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الأغذية الوظيفية هي قطاع جديد من المنتجات المصممة لتحسين الصحة. وقد ازداد الطلب على هذه المنتجات، ولاسيما منتجات الألبان الوظيفية. توفر المغذيات العلاجية العديد من الفوائد الطبية أو الصحية للمستهلكين، علاوة على تأثيرها الداعم لبعض بكتريا البروبيوتك. منتجات السينيوتك هي مزيج من بكتريا البروبيوتك والمواد الحيوية (بروبيوتك) التي تؤثر بشكل مفيد على العائل. إضافة المواد الحيوية أو أي مواد أخرى لمنتجات الألبان يمكن أن تؤثر على جودتها وخصائصها. لذا تم في هذا البحث دراسة تأثير ثلاثة تركيزات من ثلاثة عشر من المستخلصات النباتية: ثمانية مستخلصات مائية (الكليل الجبل، الشبث، الثوم، الزنجبيل، بذور الكتان، الشوفان، الزعتر، وأوراق المورينجا) وخمسة مستخلصات باستخدام الدايميثيل سلفوكسيد لإكليل الجبل، الشبث، الثوم، الزنجبيل، والكتان على عشرة سلالات بكتيريا بروبيوتك مختلفة من مجموعة بكتريا حمض اللاكتيك. أوضحت النتائج حساسية بعض السلالات البكتيرية (*Lactobacillus brevis* KP653، *Lactobacillus delbrueckii* subsp. *Lactis* KP645، and *Lactobacillus acidophilus* CHA2) نموها بينما تأثرت سلالات أخرى إيجابياً ببعض تلك المستخلصات (*Lactobacillus delbrueckii* subsp. *delbrueckii* KT615، *Bifidobacterium longum* 141B، *Lactobacillus plantarum* KP623، *Lactobacillus delbrueckii* subsp. *bulgaricus* YASH2، *Lactobacillus johnsonii* A1، and *Streptococcus thermophilus* T11) وقد تم انتاج ستة عشر عينة يوجورت سينيوتك والتي شابهت العينة القياسية أو كانت قريبة منها ولاسيما في الشكل الظاهري وقد تراوحت درجة القبول العام بين 76 الى 98%. وقد حصلت العينة القياسية على أعلى درجة من القبول العام تلتها عينات اليوجورت المحتوية على مستخلصات إكليل الجبل والشبث وبذور الكتان والزعتر والشوفان وأوراق المورينجا في حين سجل اليوجورت المحتوي على مستخلص الثوم أقل درجة قبول عام.

الكلمات الدالة: المغذيات العلاجية، البروبيوتك، بكتريا حمض اللكتيك، سينيوتك، اليوجورت