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In-Vitro Antioxidant and Antimicrobial Activities of Selected Fruit and Vegetable Juices and Fermented Dairy Products Commonly Consumed in Egypt.

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ABSTRACT

The antibacterial activities of the juices from fruits and vegetables and different fermented dairy products commonly consumed in Egypt were evaluated for their antioxidative and antimicrobial activities. A combination of biochemical analysis and bioassays to identify potency compounds included the 2,2-diphenyl-2-picrylhydracyl hydrate (DPPH) radical scavenging method and Folin–Ciocaulteau total phenol method were used to study the distribution and levels of polyphenols and antioxidants (AOXs) contents. For the antimicrobial activity analysis, seven pathogens strains bacterial inoculated (10^4 cells/ml) into 20 ml of Mueller-Hinton agar and poured in Petri dishes, using the well diffusion, were added juices of fruits, vegetables and different fermented dairy products. After incubation, antibacterial activity was measured by zone of inhibition (ZOI). The pomegranate juices were highest in their polyphenol content, antioxidant activity compared to all other juices. High significant positive relationship existed between the polyphenolic content and the antioxidant activities of the juices. Only fresh juices of lemon (4.4 mg/well) and fresh pomegranate (3.6 mg/well) had the potency to exhibited inhibition activities towards the seven studied antibiotic resistant microorganisms. Sobya was the only lactic acid fermented dairy products with high antioxidant activity and efficiently inhibited the seven studied antibiotic resistant microorganisms. Lemon and pomegranate juices and fermented sobya may be a good candidate for functional foods as well as for nutraceutical plant based products.
Keywords: Fruit and vegetable juice, fermented dairy products, Lemon juice, Pomegranate juice, Fermented sour sobya, Antioxidative activity

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INTRODUCTION

The beginning of the last century witnessed the production of antibiotics, with their potential therapeutic benefits against a wide range of microorganisms including gram-positive and gram-negative bacteria, saving the life of cases with fatal illnesses. Today, the problem of antibiotic multidrug-resistant (MDR) Gram negative bacteria such as certain strains of *Pseudomonas aeruginosa* is growing at an alarming rate [1] methicillin-resistant *Staphylococcus aureus* [2].

In the last two decades, efforts have been developed to screen, different plant species and fermented food products in various geographical regions for identifying and optimizing novel naturally–occurring antimicrobial compounds.

Sweet and bitter kernels of apricot were reported to be potentially rich sources of antimicrobial agents for *Escherichia coli* and *Staphylococcus aureus* nose [3]. The pomegranate juice had high antibacterial effect on *B. cereus*, *E. coli*, *Pseudomonas aeruginosa* and *S. aureus* [4].

The intact cells and cell-free extract of *Lactobacillus case*i subsp. *casei* SY13 and *Lactobacillus delbrueckii subsp. bulgaricus LJJ,* isolated from the traditional yogurt exerted radical scavenging ability on (DPPH) and the authors suggests that the antioxidative property of *Lactobacilli* could beneficially affect the consumer by providing dietary source of antioxidants [5]

Screening *Lactobacillus* strains including commercial probiotic ones and *L. rhamnosus GG* showed that all *Lactobaccilli* displayed strong antibacterial activity toward the Gram-negative pathogen *Salmonella enterica serovar typhimurium* SL1344 and significantly inhibited invasion of the pathogen into cultured human enterocyte-like Caco-2/TC7 cells [6]. The authors contributed the antibacterial activity of *L. rhamnosus* GG to the production of lactic acid.

Lactobacillus rhamnosus GG spent culture supernatant (LGG-SCS) was reported to have antimicrobial activity against *Salmonella enterica serovar typhimurium* (*S. typhimurium*) [7].

Diversification of the sources of probiotics to include traditional fermented foods had been addressed by scientists from different parts of the world and is a cost effective strategy [8];[9]. Kefir is a fermented milk beverage produced by the symbiotic association of diverse bacteria and yeasts and the possible associated bioactive metabolic compounds confers kefir the status of a natural probiotic, designated as the 21th century yoghurt [9]. Kefir has antimicrobial, and immunomodulatory activities. Kefir has been also postulated in the apoptotic effect in HTLV-1- negative malignant T-lymphocytes [10]

It is the objective of the present study to screen the juices of commonly consumed fruits & vegetables for their total poly phenol contents (TPC), antioxidant and antimicrobial activities. Selected fermented dairy products were also examined for their antioxidative and antimicrobial activities.

MATERIALS AND METHODS

Materials

Fresh fruits (6) and vegetables (3) were purchased in batches from the public market. The fruits were washed with tap water and the juices were collected using electric press (Braun, Germany). The juice was saved in air tight containers in the deep freezer at -20^{o}C. Commercially available apple, purple grapes and pomegranate juices in carton cans were also purchased from the retail market.

Fermented dairy products, these consisted of four commercial brands of yoghurt and commercial sobya sample. Sobya is traditional fermented rice and milk based product containing diverse lactic acid bacteria and yeasts. The porridge has a pH of 3.5 and is rich in expoysaccharides and lactic acid with added sugar and flavourings, which confers to viscosity and sour flavour. Table (1) presents the microbial count of the five fermented products.

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Table 1: The chemical composition of different fermented milk products.						
Parameters	**Unit**	**Activia yogurt**	**Juhina Yogurt**	**Juhina light Yogurt**	**Regular Yogurt**	**Sobya**
Total solids	G %	13	10.5	10.5	10.5	31.6
Carbohydrate	g	4.5	4.5	4.5	4.5	27.1
Energy	Kcal	67	57	57	57	138.4
Total *Bifidobacterium spp.*	cfu/g	1.5×10^8	$< 10^6$	$< 10^6$	$< 10^6$	$< 10^6$
Total *Lactobacilli*	cfu/g	5.11×10^9	$< 10^6$	$< 10^6$	$< 10^6$	3.0×10^9
Yeast	cfu/g	$< 10^6$	$< 10^6$	$< 10^6$	$< 10^6$	1.0×10^8
Culture of yoghurt						
Lactobacillus bulgaricus	cfu/g	6.4×10^9	6.4×10^9	6.4×10^9	6.4×10^9	--
Streptococcus thermophilus	cfu/g	3.9×10^9	3.9×10^9	3.9×10^9	3.9×10^9	--

Analytical methods

Total solids determination by the thermo gravimetric analysis Well mixed 5 g of the well mixed juice was Accurately weighed into a preheated and pre-weighed flat-bottomed glass dish containing 3 grams filter paper cut in pieces 3 x 3 millimeter and provided with a fitting lid (about 4 cm diameter).

The uncovered dish was placed overnight in the oven at 87 ^{o}C until most of the moisture was driven off. The Bottom of the dish was then wiped off and the dish was transferred to a well-ventilated oven at 102 ^{o} C.

The Lid was placed next to the dish in the oven. They were dried for 2 h in the oven and then the dish was covered with the lid, cooled for 30 min in a desiccators and weighed using sensitive analytical balance to measure weight changes.

The Dish and the lid was heated again for 30 min periods in the oven, cooled and weighed until the difference between the two successive weightings did not exceed 1 mg [11].

Total polyphenols

Accurately weighed portions (5 g) of the juice were extracted successively and vigorously in dim light with 5 ml distilled water and then with 20 ml absolute methanol [12].After centrifugation in the cold, the upper layer was aspirated and concentrated in vaccuo to 4 ml. Clean up step on Cartridge Strata-C18 (Phenomenex, Torrance, CA). The cartridge was equilibrated with the successive addition of absolute methanol (1 ml) and H_2O (1 ml). Aliquots of the juice extracts (2 ml) were loaded on the SPE, washing with the successive addition of H_2O (10 ml) and 5% aqueous methanol (1 ml). The [PP]-containing fraction was collected after the addition of 5 ml absolute methanol. The elute was concentrated under a stream of nitrogen to 2 ml. Aliquots (20 µl) were taken for the analysis of [PP] using the Folin Ciocalteu method. After the successive addition of Folin_Ciocalteu reagent (10 µl) and 20% sodium carbonate (32 µl), the final volume was brought to 300 µl with H_2O. The tubes were left in the dark for 60 min and then the absorbance of the colour was measured spectrophotometrically at 765 nm . A blank and standard working solutions of gallic acid (1-14 mg/l) were run in parallel and the results were expressed as gallic acid equivalents (GAE) per deciliter of juice.

Antioxidant activity evaluation

The antioxidant activity of the juices and fermented dairy products was evaluated by the free-radical scavenging capacity. The method assayed used 90 mM in methanol [13] of the stable free radical DPPH compound (2,2 diphenyl-1-picrylhydrazyl). The samples were prepared for the analysis without dilution or after dilution with methanol at 1:4, 1:1, 3:1 (v: v). The standard ascorbic acid solutions were used within 1 h of preparation. Aliquots of 80 µl of diluted standards or samples were mixed in an Eppendorf tube with 2000 µL of the methanolic DPPH solution and all tests were operated in triplicate. The reaction was left in the dark for 15 min under continuous stirring. The absorbance was then measured at 515 nm using methanol as blank to zero the spectrophotometer and the DPPH solution was used as the control. The results were expressed as mmol ascorbic acid equivalent antioxidant capacity (AEAC) [14].

Determination of antibacterial activity

The antimicrobial activity of 12 juices and 5 fermented dairy products was determined by the agar well diffusion method [15] The seven pathogenic indicator bacteria strains were obtained from the stock cultures of the Dairy Microbiological Lab, National Research Center: *Escherichia coli* 0157: H7 ATCC 6933, *Bacillus cereus* ATCC 33018, *Staphylococcus aureus* ATCC 20231, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, *Listeria monocytogenes* ATCC 7644 and *Yersinia enterocolitica* ATCC 9610. Each strain was activated in Tryptone soy broth by fermentation at 37 ºC for 24 h. One ml culture of the activated indicator strain (10^4 cells /ml) was inoculated into 20 ml of Mueller-Hinton agar (Becton Dickinson, USA) and poured in Petri dishes. After solidification of the agar, wells of 5 mm in diameter were cut from the agar with a sterile borer and 50µL of juices delivered in each well. To avoid the effect of the produced organic acids, fermented products were neutralized to 6.0 - 6.5 using 1 N sodium hydroxide.

Penicillin (Potassium penicillin 20 %) was used as positive reference standards to determine the sensitivity of each microbial species tested. Working penicillin solutions were prepared in increasing concentrations ranging between 5 and 20 % equivalent to 50 and 200 mg/ml. Controls were sterile phosphate buffer solution and all tests were carried out in triplicates. The plates were incubated at 37°C for 24 h.

The antimicrobial activity was expressed as the diameter of the zone of inhibition (ZOI); whereby a diameter >1mm around the well was considered as a positive result and the greater the diameter of the ZOI, the higher is the antimicrobial activity. The % inhibition was calculated according to National Committee for the Clinical Laboratory (NCCLS).

The zone diameter of wells cut in nutrient agar medium was 5.0 mm and the diameter of inhibition zone (DIZ) of negative a control for each bacterium was also 5.0mm. If the DIZ value is 5.0 mm (*), that means the sample has no inhibitory activity against that bacterium.

Statistical analysis

All tests were carried out in triplicates and the results were expressed as the arithmetic means ± standard deviation from the mean. Differences between means at the 95% ($P < 0.05$) confidence level were considered statistically significant (Student's t - test). Correlations were obtained by Pearson's correlation coefficient (r) in bivariate linear correlations.

RESULTS

The characteristics of the 12 fruit and vegetable juices are presented in Table (2). The mean total solids of the freshly prepared apple, grapes and pomegranate juices were significantly lower (P<0.05) than the respective level in the commercial juices. This is because the commercial juices were reconstituted from the respective juice concentrate as stated on the label. The juices of commercial pomegranate were the richest in [PP] content, while lemon juice is quite poor in its total [PP (Table 2).

Table (3) presents the antioxidant activity of the studied juices and fermented products. The commercial pomegranate juice had the highest antioxidant activity (6.53±1.61 mmol) ascorbic acid equivalent antioxidant capacity) but difference from the respective value of the house prepared natural pomegranate juice (4.54±0.88 mmol) didn't attain significance (>0.05). Similarly, the antioxidant activity of the commercial purple grape juice (5.03±0.92) was significantly higher (P<0.05) compared to the in house prepared natural red grapes (1.57±0.33). On the other hand, the antioxidant activity of the in house prepared apple juice (2.49±0.48) was higher than the commercial brand (1.63±0.15), yet the difference didn't attain statististical significance (P>0.05).

Table 2: List of the scientific names, total solids and total poly phenol contents (TPC) of the studied fruit and vegetable juices.

Rank	Fruit / Vegetable Juice	Scientific name	Total solids %	TPC, mg gallic acid equiv / 100 grams
				Ẋ±SE
		Fruit juices		
1	Apple, Fresh	*Malus domestica*	9.42	8.5±0.75
	,Canned	*Malus domestica*	14.50	3.4±0.38
2	Apricot, Fresh	*Prunus eriogyna*	13.87	2.3±0.04
3	Grapes, Fresh	*genus Vitis*	10.18	2.6±0.57
	,Canned	*genus Vitis*	15.80	11.2±0.61
4	Grapefruit, Fresh	*Citrus paradisi*	7.98	12.9±0.47
5	Lemon, Fresh	*Citrus limon*	8.85	3.7±0.61
6	Pomegranate, Fresh	*Punica granatum*	7.11	10.3±0.11
	,Canned	*Punica granatum*	14.00	19.2±0.09
		Vegetable juices		
1	Onion Fresh	*Allium cepa*	7.54	3.9±0.42
2	Green chilis Fresh	*Capsicum annuum*	6.71	12.8±0.19
3	Tomato Fresh	*Solanum lycopersicum*	2.87	9.8±0.19

Table 3: Mean anti-oxidant activity of the studied fruit, vegetable juices and fermented dairy products.

Rank	Food item		Antioxidant activity (AEAC)* (Ẋ±SE)
			Fruit juices
1	Apple, Fresh		2.49±0.48
	,Canned		1.63±0.15
2	Apricot, Fresh		2.85±0.37
3	Grapes ,Fresh		1.57±0.33
	,Canned		5.03±0.92
4	Grapefruit, Fresh		4.14±0.48
5	Lemon, Fresh		2.96±0.47
6	Pomegranate, Fresh		4.54±0.88
	,Canned		6.53±1.61
			Vegetable juices
1	Onion, Fresh		2.34±0.44
2	Pepper green, Fresh		3.19±0.63
3	Tomato, Fresh		2.38±0.37
			Fermented dairy products
1	Acitvia yoghurt		1.90±0.45
2	Juhina actilife		1.68±0.41
3	Juhina light		1.84±0.44
4	Regular yoghurt		2.14±0.18
5	Sobya		4.55±0.78

* mmol ascorbic acid equivalent antioxidant capacity

The antioxidative activities of the fruits and vegetables correlated significantly ($P<0.001$) with the polyphenol contents, with Pearson correlation coefficient of 0.86. Figure (1) illustrates the scatter plot for fruit and vegetable PP and the antioxidant activities and the regression equations for best fitting line.

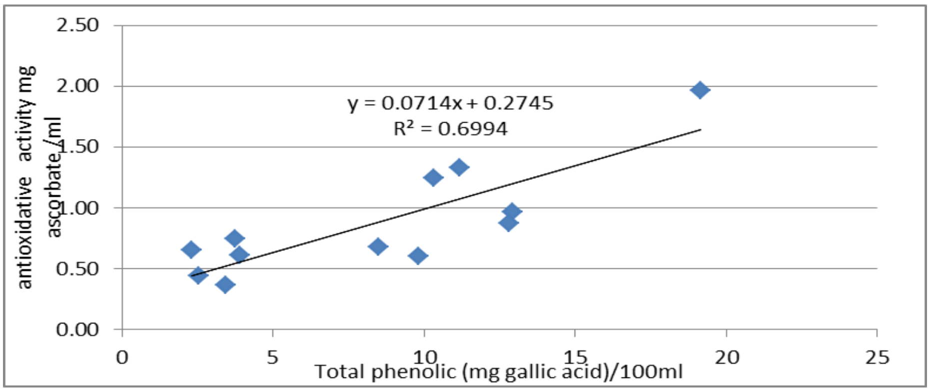


Figure 1: Scatter plot for fruit and vegetable TPC versus the antioxidant activities and the regression equation for best fitting line.

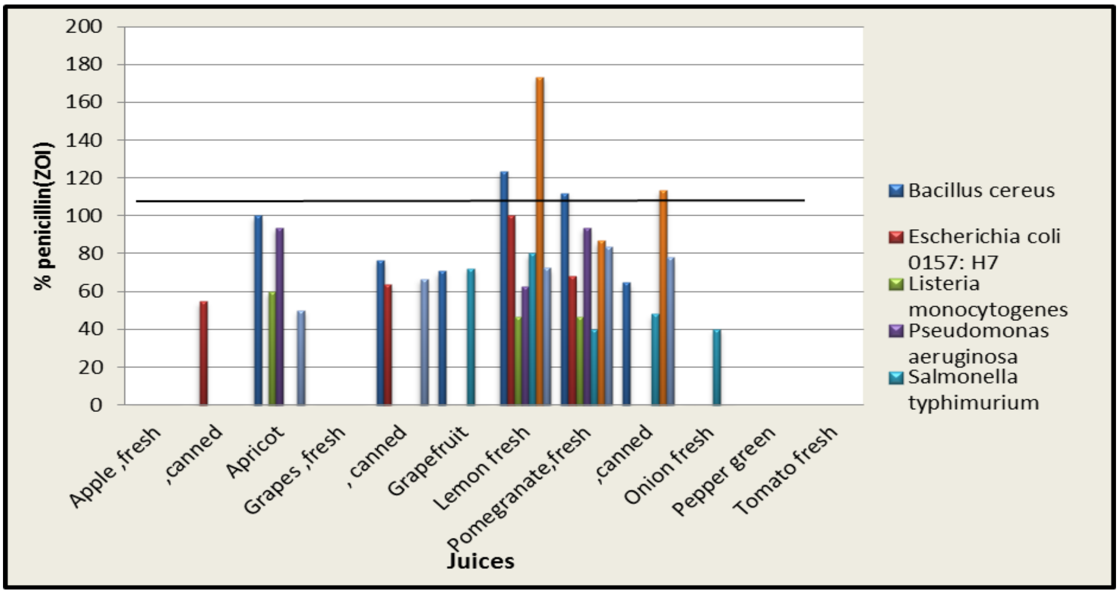
From the 5 studied fermented products, sour sobya with (4.55±0.78 mmol) ascorbic acid equivalent antioxidant capacity was highest in its antioxidant activity Table 3.

Table 4: Antimicrobial activity of fruit & vegetable juices and fermented products measured as Zone of inhibition (mm).

Samples		Pathogenic bacteria						
		Gram-negative				Gram-positive		
	mg/well	*E. coli 0157: H7*	*Pseudo monas aerugin osa*	*Salmon ella typhim urium*	*a enteroc olitica*	*Bacillus cereus*	*Listeria monocy togene s*	*Staphyl ococcus aureus*
Standard								
Penicillin	5	22	16	25	18	17	30	15
Fruit juices								
Apple ,fresh	4.7	0	0	0	0	0	0	0
,canned	7.3	12	0	0	0	0	0	0
Apricot	6.9	0	15	0	9	17	18	0
Grapes ,fresh	5.1	0	0	0	0	0	0	0
, canned	7.9	14	0	0	12	13	0	0
Grapefruit	4.0	0	0	18	0	12	0	0
Lemon fresh	4.4	22	10	20	13	21	14	26
Pomegranate,fresh	3.6	15	15	10	15	19	14	13
,canned	7.0	0	0	12	14	11	0	17
Vegetable juices								
Onion fresh	3.8	0	0	10	0	0	0	0
Pepper green	3.4	0	0	0	0	0	0	0
Tomato fresh	1.4	0	0	0	0	0	0	0
Fermented products								
Activia Bio yogurt	6.5	8	18	19	0	8	13	15
Juhina acti life	5.3	8	17	19	0	0	13	13
Juhina light	5.3	8	18	18	0	0	13	17
Regular yogurt	5.3	8	20	20	0	10	13	15
Sobya	15.8	17	10	16	7	12	15	16

Penicillin and food items with shadow denotes the inhibitory activity towards the 7 studied pathogenic microorganism

Table (4) presents the antimicrobial activities of the studied food products towards 7 pathogenic microorganisms relative to penicillin. Interestingly, freshly prepared lemon juice (4.4 mg/well) and in house prepared pomegranate juice (3.5 mg /well) were the only two juices to exert inhibitory activities on the 7 pathogenic microorganisms. While sobya was the only LAB fermented milk product with the potence to inhibit the 7 pathogenic organisms. Commercial pomegranate juice at concentration of (7 mg/well) failed to inhibit the growth of *E. coli* 0157 H7, *P. aeruginosa* and *L. monocytogenes* suggesting that pasteurization and processing of the juice is associated with the loss of a thermal labile bioactive flavonoid compound. The vegetables showed no antibacterial activity, except for onion juice, which inhibited only the growth of *Pseudomonas aeruginosa*. Figure (2) illustrates the relative antimicrobial activities of the juices and fermented foods, with the zone of inhibition for penicillin set as 100.



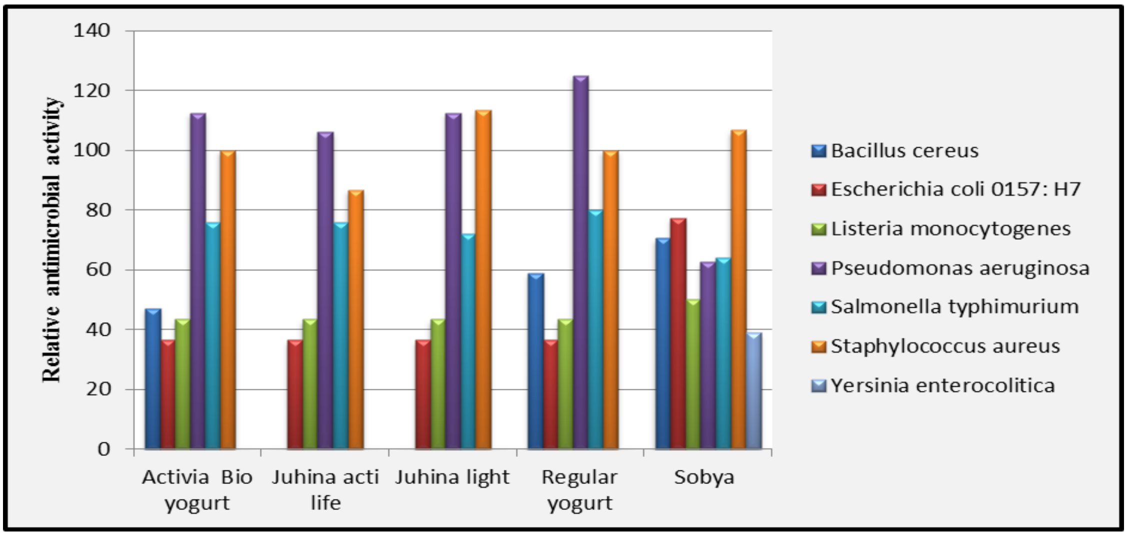


Figure 2: The relative antimicrobial activities of the juices (top) and fermented dairy products (bottom), expressed as percentage of penicillin values. Penicillin zone of inhibition (ZOI in mm) set = 100.

DISCUSSION

Fruit and vegetable flavonoids are directly associated with human dietary ingredients and health. A study was reported on the analysis of edible fruits and vegetables grown in France for their total polyphenol content [19] .Wide variation existed in the TPC within the same fruit item depending on the variety and the stage of ripening; TPC of fresh apples ranged between 90 -300 mg gallic equivalent per 100 g fresh edible fruit., Respective ranges for apricots 103 - 318; for lemon 35- 55; for grapefruit 39- 48. For fresh edible vegetables, the TPC fluctuated within narrow ranges for green pepper 18.2, for onion 76 and for tomato 10 - 26 mg gallic acid equivalent per 100 fresh edible tomato [19] .Based on the results of the French study, the local variety effect must be stressed and there is a need to evaluate structure and function relationship of food polyphenols. Fruit polyphenols responsible for free radical scavenging activity are epicatechin, hyperoside and chlorogenic acid, and these compounds are considered to be the best antilipoperoxidants[20].

Great differences were reported in the total phenolic contents of the same fruit or vegetable as reported by different authors. In the present study TPC of apricot juice averaged 2.3 mg gallic acid equivalent per 100 g. This level is quite inferior to the TPC obtained with Hungarian apricots with mean levels ranging between 4.5 - 20 mmol gallic acid equivalents per 100 ml, which are equivalent to 7.6 - 34 mg gallic acid equivalent per 100 ml [21].

This discripancy is due to differences between the analytical methodologies used the Folin Ciocalteau assay clearly detects all polyphenolics present in the sample, and other non phenolic antioxidants. In the present study all juices were subjected to clean up step with solid phase extraction cartridge (SPE - Waters) to get rid of all impurities [12].

Another study reported the TPC of water extracts of Turkish sweet and bitter apricot kernels with mean values of 0.79 and 0.04 mg per 100 ml, respectively. The respective DPPH radical scavenging activities in the same apricot extracts were 89.9 % and zero % [3]. Both extracts had similar antibacterial activities against *Escherichia coli, Staphylococcus aureus*, but the apricot extracts failed to inhibit *Enterobacter aerogenes* or *Pseudomonas aeruginosa* [3].

All fruit juices had antibacterial activities in concentrations ranging between 3.5 - 7 mg total solid per well. Lemon has been used for centuries in various societies to combat infectious disease. Our results agree with those reported earlier by [22]. The authors showed that Lime juice at a concentration of 512 mg /ml had antimicrobial activity against all 9 negative and positive gram bacteria studied.

In the present study , Lemon juice 50 µl containing 3.5 mg dry matter per well had significant bactericidal action, against a plethora of Gram-positive, Gram-negative, including resistant strains such as *Baccillus ceurus, E. coli methicillin-* and *ciprofloxacin-resistant Staphylococci, Listeria, ciprofloxacin-resistant Pseudomonas aeruginosa, Salmonella typhimerium* and *Staphylococcus aureus* and *Yersinia*. The same applied to natural pomegranate juice prepared at the laboratory.

A study showed that the ethanolic or water extracts of apple, lemon and pomegranate had antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus*; meanwhile commercially available standard *pennicillin* didn't inhibit *P. aeruginosa* [23].

In the present study, commercial pomegranate juice lost the antibacterial activities against three out of 7 tested pathogenic microorganisms; namely *E. coli*, *Listeria* and *Pseudomonas*. This finding suggests that the technological processing of pomegranate is associated with changing in the structural configuration of bioactive flavonoid molecules. More recently pomegranate *Pseudomonas, Proteus, Staphylococcus aureus, Escherichia coli, Salmonella, Bacillus subtulis* and *Clostridium* [24].

Commercial pomegranate juices were reported to contain the tannin punicalagin (1500-1900 mg/L) while only traces of this compound were detected in the experimental juice obtained from arils in the laboratory [14] contributing to the higher antioxidant activity of commercial juices compared to the experimental ones.

Among the vegetable juice tested, neither green chilis nor tomato juice had any antibacterial activities. Onion juice exerted excellent antibacterial activity against *Salmonella typhimerium*. These results are in good agreement with those reported earlier showing lowest antibacterial activity for *Salmonella typhimerium* [23].

In the present study, Sobya effectively inhibited the growth of all 7 gram positive and Gram negative bacteria. The remarkable antimicrobial attributes of the *Lactobacillus* species or yeast from sobya could be attributed to different factors, including the accumulation of main primary metabolites such as lactic and acetic acids. The inhibitory activities of LAB against Gram positive pathogens have been mostly shown to be due to the bactericidal effect of protease sensitive bacteriocins [25]. The antagonistic effects of LAB towards Gram negative pathogens could be related to the production of organic acids and hydrogen peroxide. Major

LAB in sobya is *L. rhamnosus* GG (unpublished data). The probiotic *Lactobacillus rhamnosus* GG had been reported to exert antibacterial activity against *Salmonella typhimurium* and the antimicrobial compounds consisted of low-molecular weight, heat-stable, non-proteinaceous bactericidal substance, active at acidic pH against a wide range of bacterial species ; the chemical identity remained unknown [7].

The antioxidative activity and the antibacterial activity of sobya found in the present study are quite similar to those reported previously with kefir. The fermented rice milk Kefir effectively inhibited the pathogenic bacteria *B. subtilis* and *E. coli* [26]. Moreover, the good antioxidant properties of rice milk kefir denotes to its role as useful natural antioxidant supplements for the human diet.

MRSA (*methicillin* resistant *Staphylococcus aureus*) can cause life-threatening infection and is resistant to beta-lactam antibiotics. Killing the pathogenic microorganisms. The bioactive peptides released in response to fermentation and protein hydrolysis could be lethal to the pathogenic bacteria. The pathogenic bacteria *E. coli, S. aureus, B. subtilis* tested in the present study are known to promote gastrointestinal disorders such as diarrhea, which is of public health concern.

Bacillus cereus or B. cereus is a Gram positive food borne disease has the ability to withstand time and harsh environments because it can form endospores that are resistant to heat, producing diarrheal toxins [27].

Most persons infected with *Salmonella* bacteria develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment. However, in some persons, the diarrhea may be so severe that the patient needs to be hospitalized. Salmonella infection may spread from the intestines to the blood stream and then to other body sites and can cause death unless the person is treated promptly with antibiotics Bloodstream infections caused by nontyphoidal *Salmonella* in Africa were reported in 2012 to have a case fatality rate of 20–25%. Most cases of invasive nontyphoidal *Salmonella* infection (iNTS) are caused by S Typhimurium and which is resistant to the antibiotic chloramphenicol [28]. This created the need to use expensive antimicrobial drugs in areas of Africa that were very poor, thus making treatment difficult. The variant is the cause of an enigmatic disease in sub-Saharan Africa called invasive non-typhoidal *Salmonella* (iNTS), which affects Africa far more than other continents. This is thought to be due to the large proportion of the population with some degree of immune suppression due to the burden of malnutrition, especially in children.

Infants and young children are much more susceptible to infection with Salmonella, easily achieved by ingesting foods infected with a small number of Salmonella. The organism enters through the digestive tract to reach the gastrointestinal tract and multiply in tissues (localized form). *Salmonella* has evolved a degree of tolerance to acidic environments that allows a subset of ingested bacteria to survive. By the end of the incubation period, the nearby cells are poisoned by endotoxins released from the dead *Salmonella*. The local response to the endotoxins is enteritis and gastrointestinal disorder. *Listeria* [29] and *Pseudomonas aeruginosa* [30] strains are the most resistant microorganism strains.

CONCLUSION

Lemon juice and pomegranate juice in their intact form without exposure to thermal processing and sour sobya have the potential for exploration of broader applications as good candidate for functional foods and as nutraceutical plant based products. Microencapsulation is an applicable option and awaits further investigations.

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