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Design of a New Fermented Beverage from Medicinal Plants and Organic Sugarcane Molasses via Lactic Fermentation

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Abstract: Functional beverages obtained using medicinal plants and fermented with lactic acid bacteria are gaining much interest from the scientific community, driven by the growing demand for food and beverages with beneficial properties. In this work, three different batches of medicinal plants and organic sugarcane molasses, named FB-lc, FB-sp and FB-lcsp, were prepared and fermented by using *Lactobacillus acidophilus* ATCC 43121, *Bifidobacterium breve* B632 and a mix of both strains' culture, respectively. The three fermented beverages revealed a high level of polyphenols (expressed as gallic acid equivalent), ranging from 182.50 to 315.62 µg/mL. The highest content of flavonoids (152.13 µg quercetin equivalent/mL) and tannins (93.602 µg catechin equivalent/mL) was detected in FB-lcsp trial. The IR spectroscopy analysis showed a decrease in sugar (pyranose forms, D-glucopyranose and rhamnosides). In addition, the aromatic compounds of the fermented beverages, detected by GC-MS headspace analysis, showed twenty-four interesting volatile compounds, which could give positive aroma attributes to the flavor of the beverages. The highest antioxidant activity was observed in the beverage obtained by the mix culture strains. Accordingly, the production of these beverages can be further investigated for considering their well-being effects on human health.

Keywords: lactic fermentation; functional beverages; volatile compounds; antioxidant activity

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1. Introduction

Since ancient times, medicinal plants have been used for human well-being as they are a source of healthy metabolic compounds. They still are widely used for the production of food, medicines and fuel. The improvement of the biological properties of plants, vegetables and herbs can be achieved through fermentation technology, which can transform complex substrates and change the quantity of some bioactive compounds [1]. Additionally, the fermentation process allows the preservation of food and beverages while improving the nutritional and organoleptic quality of the products [2]. Selected microorganisms are widely applied to obtain fermented foods and beverages. Among them, yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) are the main organisms with beneficial technological applications and for which a wide knowledge of use is available [3,4]. In some cases, mixed cultures are preferred over single cultures since they can offer improved processes and higher flavor and bioactive compound content [5], as well as health benefits [6]. Applying selected microbial cultures to suitable raw materials, the fermentation process can be modulated, also enhancing the content of bioactive metabolites and other compounds with antioxidant activity [7–10]. Since the production of specific metabolites is a strain-specific trait, the choice of microorganisms for the intended use is fundamental to obtain fermented products with enhanced properties [11–13].

A comprehensive inventory of microorganisms and their status as GRAS (generally recognized as safe) and/or QPS (qualified presumption of safety) organisms for the intended use is provided by Bourdichon and Casaregola [14].

In particular, LAB strains can produce key volatile compounds, active amino acids, peptides and sugars that contribute to the final flavor of fermented food products [15–17].

Moreover, due to the probiotic activity of some LAB, they can confer further attributes to fermented foods and beverages [18,19].

Plants are also suitable substrates for the growth of LAB [20–22]. Aromatic and medicinal plants are particularly valuable for selective bioprocesses as they already contain many bioactive compounds, including phenolic compounds, carotenoids, anthocyanins and tocopherols [23,24]. Moreover, the content of these compounds can be increased by the metabolic activity of the microorganisms involved in the fermentation process [25–27]. The metabolism of phenolic compounds correlated with increased antioxidant activity was observed during lactic acid fermentation [23,28].

Several authors have successfully obtained fermented products with improved antioxidant activity from vegetables, fruits, sugarcane molasses or plants [29,30]. Functional beverages that contain significant amounts of bioactive compounds can offer several health benefits. For instance, they can prevent some damaging physiological activities including metabolic and cardiovascular diseases [21–33].

Several LAB strains have been proposed as candidate probiotics due to their ability to provide functional attributes to foods and beverages.

Within the *Bifidobacterium* genus, some species are reported as potential probiotics for the treatment of enteric disorders in newborns such as infantile colics or as preventive agents for infantile diarrhea of bacterial origin. This function was also associated with strong antimicrobial activity against coliforms and other pathogenic bacteria [18]. Moreover, *Lactobacillus* species show several probiotic traits and are widely used in dairy and no dairy foods and beverages production. Among the functional attributes, those contributing to lower cholesterol levels are reported to be related to the action of strains belonging to the *Lactobacillus acidophilus* species [19]. The selection of appropriate organisms is the first step in designing and implementing bioprocesses for obtaining functional foods and beverages, as well as the availability of strain cultures in culture collections with known and validated functions [34,35].

This study aims to explore the effect of single and mixed culture of LAB strains, previously indicated as candidate probiotics, for the production of functional beverages starting with plants and organic sugarcane molasses as the raw material. The main fermentation parameters, the biochemical composition (carbohydrate, polyphenols, proteins), the volatile content and the antioxidant activity of the obtained beverages is assessed.

2. Materials and Methods

2.1. Plant Material and Fermentation

The plant material used for the formulation of three fermented plant beverages is shown in Table 1. Some plants were collected from Orbata National Park Mountain (Gafsa, Tunisia) with coordinates: N 34°22′49.8″ and E 9°3′23.4″ and others were purchased from the commercial central market of Tunisia. A voucher specimen was deposited in the Laboratory of Extremophile plants, Center of Biotechnology at the Ecopark of Borj-Cédria, and was identified by Prof. Abderrazak Smaoui. The selected plant materials were harvested and mixed with 30 g of organic sugarcane molasses solubilized in 1 L of distilled water. After the adjustment of the pH at 7.8, the solution was sterilized by pasteurization (80 °C in 15 min). Three equivalent batches were prepared and fermented using the selected strains *Lactobacillus acidophilus* ATCC 43121 and *Bifidobacterium breve* B632, provided by the Microbiology laboratory at the Centre of Biotechnology of Borj–Cédria.

Table 1. Plant material used for the fermentation process.

Plants	Botanical Family	Weight (g/L)	Used Organ	Condition
Nigella sativa	*Ranunculaceae*	5	seeds	Dry
Foeniculum Vulgare	*Apiaceae*	28	seeds	Dry
Ficus indica	*Cactaceae*	90	fruits	Fresh
Linum usitatissimum	*Linaceae*	10	seeds	Dry
Vitis vinifera	*Vitaceae*	5	seeds	Dry
Lavandula multifida	*Lamiaceae*	18	leaves	Fresh
Periploca laevigata	*Apocynaceae*	10	root	Fresh
Thymus hirtus sp. algeriensis.	*Lamiaceae*	33	leaves	Fresh
Zingiber officinalis	*Zingiberaceae*	20	root	Fresh

Specifically, a single culture of each strain, *L. acidophilus* ATCC 43121 and *B. breve* B632, was used to ferment batches named FB-lc and FB-sp, respectively. In addition, a mixed culture from both the strains was prepared and used to ferment the batch named FB-lcsp. The LAB cultures were previously grown on de Man Rogosa Sharpe (MRS broth, Oxoid, UK) at 37 °C for 24 h. Then, 10 mL of each culture inoculum with a concentration of 6 log cells/mL was added to each batch. After 30 days of fermentation, the three beverages were sterilized by filtration (0.2-µm filters) and preserved at +4 °C until analysis.

2.2. Fermentative Parameters

Titratable acidity (TA) was measured by titration using 0.1 mol/L NaOH solution and phenolphthalein as an indicator. The pH was detected by a digital potentiometer. The evaluation of reducing sugars was assessed using the dinitrosalicylic acid (DNS) method described by Warwick et al. [36]. Total proteins were measured using Bradford methods and the free amino acid according to the method described by Hermosín et al. [37]. Viable cell counts were made by inoculating 0.1 mL of samples, subjected to serial dilution in phosphate buffered saline (PBS), on MRS agar plates. Bacteria colonies were counted after 2 days of incubation at 30 °C.

2.3. Total Phenolic Content

According to the Folin–Ciocalteu method described by Tlili et al. [38], the total phenolic content was detected. A volume of 0.5 mL of Folin–Ciocalteu reagent and 1.25 mL of Na_2CO_3 (7% *w*/*v*) were added to 0.125 mL of each fermented beverage. The absorbance of each sample was measured at 765 nm after incubation of the tubes for 90 min in the dark. The total polyphenols content was expressed as µg gallic acid equivalents per mL of fermented beverage (µg GAE/mL).

2.4. Total Flavonoid Content

The total flavonoid content was assessed according to the method reported by Dewanto et al. [39]. Total flavonoids, expressed as µg of quercetin equivalent per mL of fermented beverage (µg QE/mL), were estimated concerning the quercetin standard curve (concentration range: 100–750 µg/mL).

2.5. Condensed Tannins Contents

The condensed tannins were assessed according to the method reported by Sun et al. [40]. The number of condensed tannins, expressed as µg catechin equivalent per mL of fermented beverage (µg CE/mL), was measured using a standard calibration curve.

2.6. Polysaccharides Analyses

2.6.1. Extraction

The polysaccharides dissolved in the filtrate obtained from the fermented beverages were precipitated by adding absolute ethanol (four times the volume of the filtrate) and were incubated at 4 °C for 24 h. The solution was then centrifuged at 4500× *g* rpm for

10 min. The precipitate obtained was collected and again dissolved in 20 mL of distilled water deproteinized with 2 mL of Sevag reagent (chloroform/butanol 4: 1, v/v) according to the method described by Navarini et al. [41]. Finally, the solution obtained was dialyzed using a dialysis membrane (2 µm) against bi-distilled water.

2.6.2. Infrared Spectrum Analysis

The organic functional groups were investigated by Fourier transform infrared (FTIR) analysis, which was performed using an FTIR spectrophotometer (Bruker, Ettlingen, Germany) with a spectral range of 4000–400 cm^{-1} [42]. Briefly, 2 mg of each sample of polysaccharides was diluted with 200 mg spectroscopic grade potassium bromide (KBr) powder, ground and pressed into 1 mm pellets.

2.7. Headspace Solid-Phase Microextraction of Volatile Compounds

The volatile compounds were analyzed by Headspace Solid-Phase Microextraction (HS-SPME) coupled with Gas Chromatography–Mass Spectrometry (GC–MS) as previously described by Tian et al. [43]. The GC–MS headspace analysis was performed with an iron sources temperature of 240 °C and an ionization voltage of 70 eV. The mass spectrometer was operated in scan mode from m/z 50 to 350. Peak areas were determined for each compound by integrating a selected ion unique to that compound. The Kovats retention index (RI) was calculated with a homologous series of n-alkanes (C6-C28) under the same conditions applied for the sample analyses. The volatile compounds (OAV > 1) were considered to contribute to the aroma of fermented beverages [44,45].

2.8. Antioxidant Activity Determination

The antioxidant activities of different fermented beverages were determined by the following tests. The α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method was performed according to Tlili et al. [38]. The reducing power was determined using the method described by Oyaizu [46]. The appearance of the blue green color was measured at 700 nm, vitamin C was used as the control. The total antioxidant capacity of each extract was estimated using the method described by Prieto et al. [47]; the absorbance was detected at 695 nm. Moreover, a 2,2-azino-bis diammonium ($ABTS^{\bullet+}$) radical scavenging assay was assessed using the method described by Hayouni et al. [48]. A total of 25 µL of the fermented beverages or the standard trolox was added to 2 mL of diluted $ABTS^{\bullet+}$ solution and the absorbance was measured at 734 nm. The scavenging ability was expressed as IC_{50} (µg/mL), which is defined as the concentration inhibitive at 50% of free radical scavenging.

2.9. Statistical Analyses

All the experiments were performed in triplicate. The results were reported as mean values of three replicates ± the standard deviation (sd). The differences between the average variables were considered significant at $p < 0.05$. XLSTAT statistical software version 2016 (Addinsoft, New York, USA) was used for data processing.

3. Results and Discussion

3.1. Fermentation Trials

The colony counts of the selected LAB cultures, starting from the value of 6.5 log cfu/mL, increased during the 30 days of the fermentation trials. The highest value of 12 log cfu/mL was reached in the samples fermented with the mixed culture (FB-lcsp), after 20 days of fermentation. Later, the LAB population decreased, and the final values detected were 11, 10 and 8 log cfu/mL for FB-lcsp, FB-lc and FB-sp, respectively (Figure 1a). A substantial decrease of the pH and a corresponding increase of the titratable acidity was observed during the fermentation process (Figure 1b). The lowest pH value (3.7) and the highest TA value (3.4 mL NaOH 0.1N) were assessed for the FB-lcsp samples.

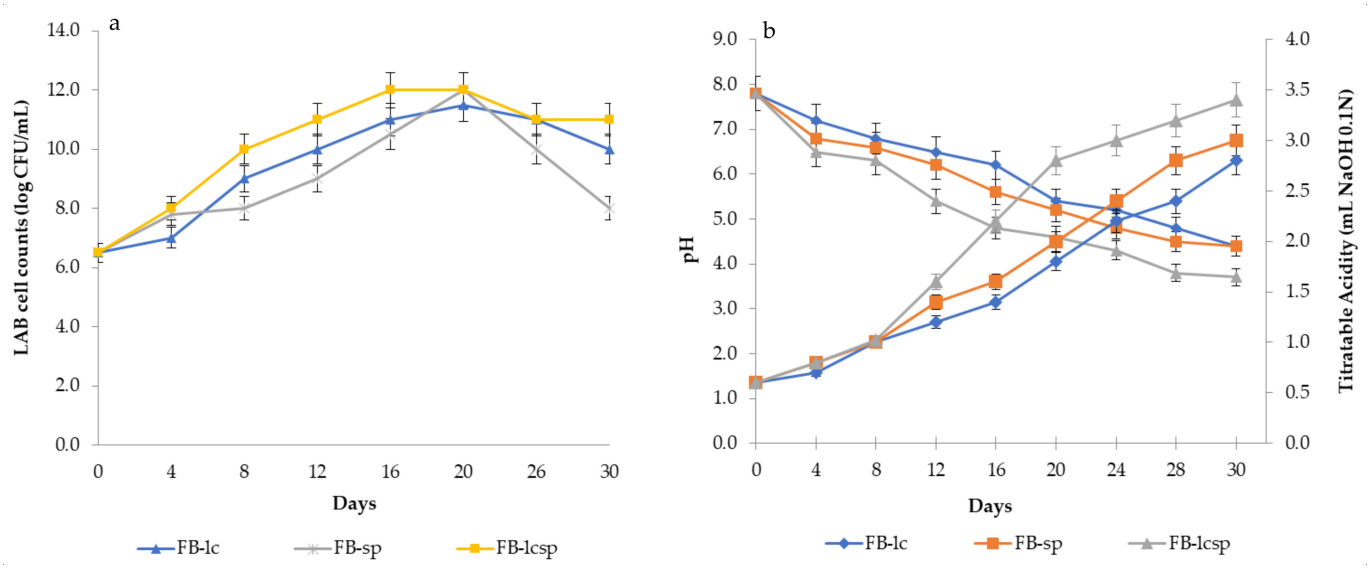

Figure 1. LAB cells count, expressed as log of colony forming unit/mL (**a**), pH and titratable acidity (**b**) of the three different products during the lactic acid fermentation process. Results are reported as mean values of 3 replicates.

A decrease of total and reducing sugars was observed, probably due to the acid production by the LAB. In addition, a degradation of the total proteins and a decrease of the free amino acid content occurred (Table 2). These results demonstrate the effectiveness of the fermentation, confirming the degradation of sugars and proteins by the LAB and the production of organic acids and new metabolites. It is interesting to note that the variation of all the considered parameters was higher for the trial obtained from FB-Lcsp, due to the combined effect of *B. breve* B632 and *L. acidophilus* ATCC 43121 cultures. During the fermentation process, the soluble components of proteins, carbohydrates and free sugars were readily available, and the consumption of these nutrients by LAB led to a drop in pH levels [49]. Moreover, the beverage was obtained by selecting hetero-fermentative LAB results that had a sparkling attribute, due to CO_2 produced, which is desired by consumers [49].

Table 2. Total sugars and proteins, total phenolic compounds, flavonoids and tannins contents of obtained beverages.

Chemical Parameters	Batch		
	FB-lc	**FB-sp**	**FB-lcsp**
Total sugars (mg/L)	23.7 ± 2.2	22.1 ± 3.3	20.3 ± 0.5
Reducing sugars (mg/L)	12.5 ± 1.4	10.3 ± 0.8	9.5 ± 0.4
Total Protein (mg/L)	17.8 ± 0.5	16.63 ±1.4	15.6 ± 0.3
Total phenolics (µg GAE/mL) *	182.5 ± 12	289.7 ± 11	315.6 ± 21
Flavonoides (µg RE/mL) **	95.2 ± 8.2	119.3 ± 2	152.1 ± 5.3
Tannins (µg CE/mL) ***	87.6 ± 7.11	73.1 ± 3	93.6 ± 9.1

FB-sp: obtained beverage fermented by *Bifidobacterium breve B632*. FB-lcsp: the obtained beverage fermented by a mix of both strains. FB-lc: the obtained beverage fermented by *L. acidophilus* ATCC 43121. * µg of gallic acid equivalents per mL of beverages. ** µg of rutin equivalent per mL of beverages. *** µg of catechin equivalent per mL of beverages. Results are expressed as the mean of 3 replicates.

3.2. Polyphenol, Flavanoids and Condensed Tannins Content

The polyphenols represent the major group of antioxidants of plant materials [50,51]. They include a wide variety of bioactive metabolites that contain at least one aromatic-hydroxyl group in addition to other substituents with large spectra of biological activities [45]. They are divided into different groups such as flavonoids, tannins and phenolic acids [45]. In this study, the amount of total phenolics, flavonoids and condensed tannins varied in the three beverages (Table 2). A high level of polyphenols, with values of 315.6;

289.7 and 182.5 µg GAE/mL, were observed for FB-lcsp, FB-sp and FB-lc, respectively. The highest content of flavonoids (152.1 µg RE/mL) and tannins (93.6 µg CE/mL) was found in the FB-lcsp trial. It is well known that the secondary metabolites possess a wide range of biochemical and pharmacological properties [48]. These results suggest that the mixed culture improves the extraction of secondary metabolites such as flavonoids, tannins and phenolic acids. During fermentation, many changes of composition occur, leading to a modified ratio of nutrients and anti-nutrients and, therefore, the properties of the product, such as bioactivity and digestibility, are modified [52]. Lactic fermentation of plants has been shown to increase the concentration of several phenolic compounds [53]. So, the richness of our fermented plant beverages with phenolic compounds, especially FB-lcsp, could be an important starting point to explore their biological activities.

3.3. Infrared Spectrum

The infrared spectrum was used to identify the polysaccharides by detecting the stretching vibration of related groups, which allows identification of a variety of functional groups, the type of sugar and glycosides. Figure 2 shows the FTIR spectra with the characteristic absorptions of the beverages before and after fermentation.

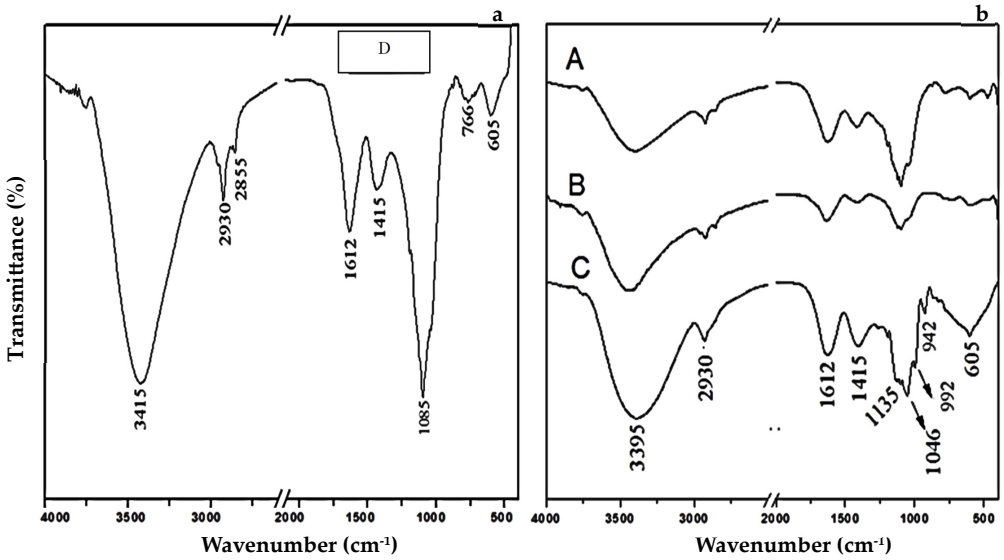

Figure 2. Infrared spectrum of polysaccharides, recorded for wavelengths 500–4000 cm^{-1}. D refers to the polysaccharides detected in not fermented beverage (**a**). A, B, and C refer to the polysaccharides detected in the beverages fermented with the selected cultures FB-sp, FB-lcsp, and FB-lc, respectively (**b**).

Substantial differences between the spectra were, indeed, observed, and the intensity of the peaks was higher before the fermentation (Figure 2a) and decreased in the fermented beverages (Figure 2b), indicating an almost total degradation of sugars.

In particular, the infrared spectrum analysis of the polysaccharides, recorded for wavelengths 500–4000 cm^{-1}, showed an intense peak between (3415–3395 cm^{-1}), representative of the vibrations of the hydroxyl groups (-OH), followed by a weak vibration of the C-H band at 2930 cm^{-1} [54]. Peaks with vibration bands between (3415–2930 cm^{-1}) are unidentified organic groups (Figure 2a). The peak at 1612 cm^{-1} is attributed to the vibration of the C = O group in its protonated carboxylic form. The peak at 1415 cm^{-1} is attributed to the vibration of the COO- group in its deprotonated carboxylic form polysaccharide extract [55]. Moreover, the peaks detected at 1200–1000 cm^{-1} indicate that the fractions are pyranose forms of carbohydrate [56]. The peaks with vibration bands at 992 cm^{-1} and 942 cm^{-1} (Figure 2b) derive from the antisymmetric stretching of tetrahydropyran, suggesting the presence of an arrangement in the carbohydrate units [57]. The peak at 605 cm^{-1} is related to D-glucopyranose. Additionally, the vibration at 766 cm^{-1} is related to rhamnoside present in the polysaccharide. The functional groups, such as –OH, –COOH

and C=O, largely found in carbohydrate in addition to anionic and cationic groups, have a good ability to counteract oxidative stress, which is expressed by excessive production of reactive oxygen species [58].

3.4. Volatile Compounds

Twenty-four volatile compounds were identified with remarkable differences between the three beverages (Table 3). Among the volatile compounds detected, some are typically produced by plants and other compounds are generated by the LAB metabolism [59]. The data showed high variations in aroma compounds. Some compounds were present only in FB-sp (1-pentanol 2-methyl, and 2-butyl acetate), and others were detected only in FB-lcsp (isoamyl alcohol, propanoic acid- 1-methylpropyl ester, α-pinene, camphene, l-phellandrene, and α-terpinene). Lactic acid, acetic acid, propyl ester, propanoic acid, propyl ester; 1,8-cineole and fenchone were also found in the three beverages. During fermentation, many changes of composition occur, leading to a modified ratio of nutrients and anti-nutrients and, therefore, the properties of the product, such as bioactivity and digestibility, are modified [60]. Among volatile compounds, alcohols are considered the principal compounds in the aromatic profile of fermented plant beverages and are a common result of the metabolism of carbohydrate and amino acids [61]. Alcohols can represent essential preservative and aromatic components in fermented plant beverages. Moreover, they can also be used as a solvent for other volatile compounds and, thus, can contribute more to the global flavor [62]. The presence of ester compounds in the fermented beverages due to the esterification of free acids with alcohol was also observed. Several volatile components in fermented plant beverages can contribute to sweet aromatic notes [63]. Our results suggest that the mixed culture of LAB could provide a suitable aroma to the fermented plants beverages.

Table 3. Aromatic compounds identified from fermented beverage extracts of by GC–MS head space.

Compounds	Ret Time	Type	FB-lc		FB-sp		FB-lcsp	
			Area	Area %	Area	Area %	Area	Area %
acetic acid, ethyl ester	3.179	BV	NF	NF	3,714,514	2.94	42,368,725	7.472
butyraldehyde, 2-methyl-	3.631	BB	3,166,922	2.372	3,165,840	2.50	3,259,781	0.574
2-amino-1,3-propanediol	4.085	BV	NF	NF	3,512,197	2.78	24,311,405	4.287
n-propyl acetate	4.282	PV	2,752,332	2.062	3,065,189	2.42	112,465,982	19.83
1-butanol, 3-methyl-, formate	4.676	BV	2,205,104	1.652	2,365,918	1.87	8,500,143	1.499
propanoic acid	4.975	PV	1,216,092	0.911	1,305,607	1.03	9,172,563	1.617
propanoic acid, propyl ester	5.857	PV	724,850	0.543	832,916	0.66	5,365,518	0.946
2-oxopentanedioic acid	5.996	BV	1,244,074	0.932	2,245,910	1.78	NF	NF
lactic acid	7.841	BV	566,468	0.424	9,635,316	7.62	2,134,730	0.376
alpha-pinene	8.138	VV	480,350	0.359	526,813	0.42	12,589,365	2.220
camphene	8.414	VB	449,670	0.336	563,489	0.45	6,925,660	1.221
α-phellandrene	9.404	PV	NF	NF	448,923	0.00	13,479,075	2.377
alpha-terpinene	9.633	BV	841,209	0.630	1,935,872	0.36	9,501,040	1.676
p-cimene	9.790	VB	28,89,327	2.164	2,889,327	0.00	18,702,987	3.298
D-limonene	9.879	BV	NF	NF	125,683	1.53	10,850,613	1.914
eucalyptol	9.950	VB	82,174,283	61.569	45,227,359	2.29	8,296,759	1.463
gamma-terpinene	10.522	PV	887,853	0.665	889,853	0.10	2,486,838	0.438
terpinolene	11.298	VV	2,302,629	1.725	2,302,629	35.77	159,646,370	28.158
fenchone	11.300	VV	NF	NF	NF	0.70	29,477,277	5.199
linalol	11.554	BB	604,976	0.453	20,304,384	1.82	36,435,120	6.426
camphor	12.844	BB	12,301,015	9.216	12,303,015	9.73	8,640,472	1.523
endo-borneol	13.341	PV	3,391,528	2.541	4,485,728	16.06	28,204,267	4.975
terpinen-4-ol	13.556	VB	15,049,231	11.275	3,263,980	9.73	14,150,705	2.496
α-terpineol	13.885	VB	218,307	0.163	1,318,397	3.55	NF	NF

FB-sp: beverage fermented by *Bifidobacterium breve B632*. FB-lcsp: beverage fermented by a mix of both strains. FB-lc: beverage fermented by *Lactobacillus acidophilus* ATCC 43121. NF: not found. BV: baseline and valley points (start at a baseline point, end at a dropline from a valley point). VB: Valley dropline and baseline points. PV: penetration point and valley point. BB: start at a baseline point, end at a dropline baseline point. VV: start and dropline at a valley point.

3.5. Antioxidant Activity

The metabolization and depolymerization of phenolic compounds, correlated with increased antioxidant activity, have been observed during lactic acid fermentation [64]. In this study, the results of total antioxidant capacity (TAC) showed a similar motif to secondary metabolite contents. The TAC differed significantly among the strains used (Table 4). The three beverages showed high antioxidant activity, in particular, FB-lcsp (82.69 µg GAE/mL). Lower values were detected in samples FB-sp and FB-lc with 73.2 and 64.83 µg GAE/mL, respectively.

Table 4. Total antioxidant capacity and the free radical scavenging activity of DPPH, ABTS and the reducing power essay of the three fermented beverages.

Antioxidant Activity	FB-lc	FB-sp	FB-lcsp	BHT	Trolox	Vitamin C
Total antioxidant capacity (µg EGA/mL)	64.8 ± 0.6	73.2 ± 2.4	82.6 ± 6.3			
DPPH (µg/mL)	17.8 ± 1.6	16.5 ± 3.1	13.4 ± 8.3	11.8 ± 2.6		
ABTS (µg/mL sample)	24.6 ± 1.6	19.3 ± 1.1	17.4 ± 0.96		10.2 ± 1.5	
Reducing power (µg/mL)	55.9 ± 4.8	48.1 ± 4.1	36.1 ± 3.1			12.5 ± 2

The scavenging ability was expressed as IC_{50} (µg/mL). The reducing power activity (FRPA) was expressed as EC_{50} (µg/mL). Results are expressed as mean of 3 replicates ± standard deviation.

The DPPH scavenging activity of the fermented beverages also varied among the fermentation trials (Table 4). Samples of FB-lcsp showed the highest antioxidant activity, compared to FB-sp and FB-lc. The free radical scavenging activity $ABTS^{\bullet+}$ of each sample was also different (Table 4). This activity was higher in samples FB-lcsp (IC_{50}; 17.4 ± 0.96 µg/mL) compared to those detected in FB-sp and FB-lc samples (IC_{50}; 19.3 ± 1.1 and 24.6 ± 1.6 µg/mL, respectively).

Regarding the reducing power activity (FRPA) of fermented beverages, expressed as EC_{50} (µg/mL), all the beverages were able to lower Fe^{3+} to Fe^{2+}, but the results differed among the three samples. Specifically, the highest FRPA was detected in FB-lcsp samples (Table 4). A significantly lower activity was found in FB-sp and FB-lc samples, indicating that the double culture was able to enhance and improve the antioxidant activity in the fermented beverages more than the monoculture. Indeed, it is well known that the total antioxidant capacity is essentially due to the presence of phenolic and bioactive compounds. Several studies reported that the polyphenols present in fermented beverages possess high scavenging activity against oxidative stress [65–67]. Recently, fungal fermentation has been used to increase the antioxidant and anti-cholesterolemic activities of food and medical compounds as well as for food preservation [68]. Moreover, many bioactive compounds, formed during seaweed fermentation, have been reported to scavenge free radicals and reactive oxygen species [69].

4. Conclusions

In this study, medicinal plants and organic sugarcane molasses were tested as raw materials to produce functional beverages via lactic fermentation. *L. acidophilus* ATCC 43121 and *B. breve* B632 were selected and used in single and multiple cultures. In particular, the strain *B. breve* B632 is widely described in the literature as a potential probiotic that is useful for the treatment of enteric disorders in newborns. Strains belonging to the *L. acidophilus* species are also valuable for functional attributes among which is the ability to lower cholesterol level.

In our work, the three fermented beverages revealed an enhanced level of polyphenols, flavonoids and tannins, especially those obtained by using the mixed culture FB-lcsp. In addition, the detection of aromatic compounds in the fermented beverages showed twenty-four interesting volatile compounds, which could give pleasant-positive aroma attributes to the flavor of the beverages. The highest antioxidant activity was observed in the beverage obtained by the mix culture strains. This innovative approach, based on

lactic fermentation performed by a mixed LAB culture, can generally be used to ameliorate secondary metabolites production and extraction from plant materials.

Accordingly, the assessment of selected strains that are able to efficiently ferment medicinal plants and organic sugarcane molasses is a crucial step for designing new functional beverages with well-being effects on human health.

Author Contributions: H.G.: conceptualization, methodology, original draft; E.A.H., E.M.-R., and M.S.T. designed the study and performed the experiments. H.G., M.G., and L.D.V., contributed to drafting the manuscript, validation, data curation, visualization, writing. All authors have read and agreed to the published version of the manuscript.

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