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ORIGINAL ARTICLE

Biological and functional properties of vine leaves

Adiba Benahmed Djilali [1,2]*, Abdelouahab Benseddik [3], Halima Boughellout [4], Karim Allaf [5], Mohamed Nabiev [6]

1 Faculty of Biological and Agricultural Sciences, Mouloud Mammeri University of Tizi-Ouzou, Algeria
2 Research Unit Laboratory, Materials, Processes & Environment (UR-MPE) in M'Hamed Bougara Univesity of Boumerdès
3 Unité de Recherche Appliquée en Energies Renouvelables, URAER, Centre de Développement des Energies Renouvelables, CDER, Ghardaïa, Algeria
4 Laboratory of Food Engineering Feeding, Nutrition and Agri-food technologies Institute (INATAA) Frères Mentouri Constantine 1 Univ. Constantine, Algeria
5 Laboratory of Engineering Science for Environment LaSIE-UMR-7356CNRS, La Rochelle University, France
6 Laboratory of Petrochemical Synthesis FHC, M'Hamed Bougara University of Boumerdès, Algeria

Abstract

Aims: The main objective of the present research work was to evaluate the (phytochemical, biochemical, and antimicrobial) properties of Muscat of Alexandria leaf powder and develop new functional dairy product using the mixture lactic bacteria and vine leaf powder as prebiotic for health applications (gastric and cardiac problems, etc.). **Material and Methods:** Various nutritional parameters of the vine leaf powder namely: pH, acidity, water content, ashes, salts, fatty acids) were determined. Also, their bioactive substances (TPC, total flavonoids content, tannin content, soluble-water polysaccharides) were extracted and quantified using referenced methods. The evaluation of antimicrobial activity of these substances was carried out by disc method. Vine leaf powder and aqueous extract were used to improve acidification kinetic. Also, functional yogurt using the mixture (lactic bacteria and vine leaf powder as prebiotic) was prepared. **Results:** The main results demonstrate that, the vine leaf powder contains high-value components such as salts with a high k/Na ratio, fatty acids (palmitic, linolenic and oleic) and bioactives (polyphenols, tannins and polysaccharides). The antimicrobial activity of these bioactive metabolites varies depending on the resistance of the strains tested. On the other hand, vine leaf TPC and polysaccharides act as an antifungal against (*C. albicans* and *A. niger*) and increase the acidification rate and consequently the growth and activity of the lactic bacteria in the yogurt, which suggests a probable prebiotic effect. **Conclusions**: Through this study, we have demonstrated the high content of vine leaves in several bioactive compounds such as polyphenols, flavonoids, tannins and polysaccharides. These compounds display an interesting antimicrobial activity and an extensive effect on the activity of lactic bacteria, which suggests a prebiotic effect.

Keywords: Bioactive substances, antimicrobial activity, prebiotic, vine leaves.

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1 Introduction

Plants and their derivatives are potentially productive sources of highly secondary metabolites such as terpenoids, tannins, alkaloids, flavonoids, etc., that may be a basis of developing both drugs [1, 2] and dietary supplements for human consumption [3-6].

Among these natural resources, vine is one of the mostly cultivated plants in the north of Algeria [7]. According to FAO estimate, the Algerian vineyard covers an area of 69.220 ha for a production of 650.000 tons [8].

The vine belongs to the family of Vitaceae (Ampelidaceae). Vine and grape have long been used in traditional medicine for their beneficial therapeutic effects against diseases related to venous insufficiency, varicose veins, capillary fragility, inflammation, chronic diarrhea. Some researchers highlighted the effect of vine leaf extract on cardiovascular diseases [9].

According to the European Medicines Agency, the different parts of the red vine (leaf and fruit) have anti-inflammatory, antimicrobial, antioxidant and diuretic properties [10].

It should be noted that vine leaf is used to prepare traditional dishes in order to treat gastric disorders and abdominal pain in many Arab countries and Turkey.

The medicinal and curative benefits of this plant can be attributed to the presence of phytochemicals and nutraceuticals such as phenolic compounds including resveratrol in particular[11].

Besides, polysaccharides are widely used in traditional pharmacopeia [2, 12]; according to Rabu and Gibson [13], they have an influence on intestinal microorganisms that metabolize dietary components, especially the non-digestible oligosaccharides that possess many beneficial physiological effects; reducing intestinal pH, inhibition of pathogen growth in gastrointestinal tract, decreasing insulin response and blood lipids levels [11, 12].

Latest studies have focused on the application of bioactive substances (peptides and polysaccharides derived from plant) in the production of dairy products to address biological or technological challenges such as the incorporation of fibers as a

* **Corresponding author:** Adiba Benahmed Djilali, Faculty of Biological and Agricultural Sciences, Mouloud Mammeri University of Tizi-Ouzou, 15007, Algeria, Research Unit Laboratory, Materials, Processes & Environment (UR-MPE) in M'Hamed Bougara Univesity of Boumerdes. Tel: (+213) 775 877 909, E-mail: adiba.benahmed@yahoo.fr

texturing agent in yogurt [14], carob extract in ice cream [15], and jujube powder as a prebiotic component in the production of functional yogurt [5].

Recently, the pectin type water extractable polysaccharides from *Plantago major* L. gained greater attention as a prebiotic that stimulates the growth of some Lactobacillus strains [16]. The analysis of phytochemicals and nutraceuticals of the vine leaf extract reveals the presence of phenolic compounds and polysaccharides. These compounds are involved in lactic acid fermentation. Choi *et al.* [17] reported that heat treatment and the fermentation temperature promote phenolic compounds, induce the release of phenolic acids responsible for the antioxidant activity, and promote the yogurt stability during the storage period.

To the best of our knowledge, no prior studies have ever been published on the development of dietary and pharmaceutical products such as prebiotic using vine plant. To produce beneficial effects inside the gastrointestinal tract (GI), probiotic microorganisms must be able to survive and metabolize under the combined action of prebiotics. Furthermore, lactose intolerance and cholesterol content are two major drawbacks associated with fermented milks [18].

The main objective of the current study was to evaluate the phytochemical, biochemical, and antimicrobial properties of Muscat of Alexandria leaf powder and develop new functional dairy product using the latter as a prebiotic.

2 Material and Methods

2.1 Plant material

The vine species used in this research is the Muscat of Alexandria, which has several appellations: Muscat Romain (Roman Muscat) or Grosgrain Muscat or large-seed Muscat. This variety comes from Egypt, and is found all over the world, except for Asia (without Japan).

2.2 Biological material

Two bacterial strains (*Escherichia coli* ATCC 25923 and *Staphyloccocus aureus* ATCC 25322), *Aspergillus niger* mold and *Candida albicans* yeast were used to test the antimicrobial activity of the bioactive substances (Total Polyphenols Compounds (TPC), tannins and water-soluble polysaccharides) extracted from vine leaves. These strains were provided by the microbiology laboratory of Mouloud Mammeri University of Tizi-Ouzou.

A probiotics mixture of two different pure freeze-dried commercial strains specifically, yogurt microorganisms *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (CHRISTAN HENCEN, Denmark) was the subject of this research.

2.3 Methods

2.3.1 Chemical characterization of vine leaves

Muscat of Alexandria leaves was manually harvested from the Timizart village of Tizi-Ouzou region (Algeria). The experiment was conducted from May to October 2018.

During sample collection, vine leaves were harvested when fully mature. The collected vine leaves were cleaned and then dried under standard air conditions at 35±2°C for 15 to 20 days. The fully dried material with 0.02 - 0.04 g H2O/g db (dry basis) moisture content was ground using an electric mill and the obtained powder was sifted using a sieve sized 500µm. The sieve type is EUROMATEST-SINTOO, NFX11-501. The final powder was stored in bags under ambient conditions until use.

Figure 1 shows the particle size distribution of vine leaf powder investigated by means of a Malvern laser diffraction granulometry device (MASTERSIZER 2000).

Phytochemical and biochemical analyses were carried out to determine various nutritional parameters of the vine leaf powder (Table 1) namely: pH, acidity, water content, ashes, salts, fatty

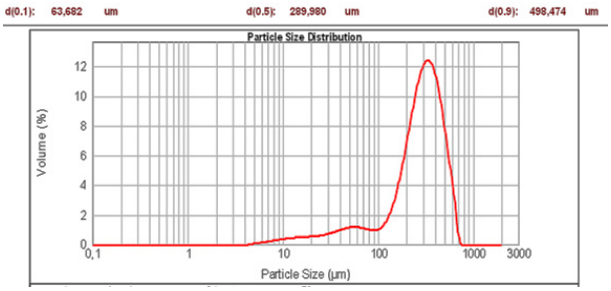

Figure 1: Granular distribution of Muscat of Alexandria leaf powder

Table 1: Physical and chemical composition of vine leaves (n=3)

Parameters		Content
pH at 20°C		4.70±0.27
Humidity (%)		10.74 ± 1.05
Ashes (%)		01.88 ± 0.02
Acidity (g/100g)		11.84 ± 0.15
SWP (%)		2.66 ± 0.05
Tannins percentage (%)		7.00± 0.02
TPC (mg AGE/g db)	**AE**	17.57 ± 0.22
	EE	16.27 ± 0.13
Total Flavonoids (mg QE/g db)	**AE**	10.23 ± 0.01
	EE	12.55 ± 0.07
Sugars (g/100mL)	**Total sugars**	3.37 ± 0.013
	Reducing sugars	0.23 ± 0.001
	Saccharose	2.98 ± 0.01

AE: Aqueous Extract; EE: Ethanolic Extract; TPC: Total Phenolic Compounds; SWP: Stem water potential.

acids, TPC, total flavonoids content, tannin content, polysaccharides as described below.

- A phytochemical analysis was made according to the standard phytochemical screening methods;
- The pH, acidity, and dry matter content were determined according to conventional methods;
- The ashes were acquired by calcinating at 550°C for 5 h a dried sample in a muffle furnace (NABERTHERM B170) according to the method described by ISO 5509 [19];
- The chemical composition (Na, K, Cr, Cd) was determined by Atomic Absorption Spectroscopy (A.A.S) method using an Atomizer (VARIAN AA 240, Australia) assisted with flame atomizer GTA 120. This method consists of dissolving 1g of ashes in 5 mL of HCl acid (0.5N) [20];
- The fat content was determined using the hexane extraction method. The fatty acids (Table 2) were determined by gas chromatography using CHROMPACK CP 9002. The methyl esters were obtained by trans-esterification in methanolic solution of potassium hydroxide with the method put forward by ISO 5509 [19].

Table 2: Fatty acids (%) of Muscat of Alexandria leaves

Fatty Acids	Names	Content (%)
C14 :0	Myristic A.	5.96
C16 :0	Palmitic A.	26.81
C16 :1ω7	Palmitoleic A.	6.43
C17 :0	Margaric A.	6.43
C18 :0	Stearic A.	4.83
C18 : 1ω9	Oleic A.	6.45
C18 : 2ω6	Linoleic A.	5.93
C18 : 3ω3	Linolenic A.	12.03
C20 :0	Arachidic A.	1.54
C20 : 1ω9	Gondoic A.	6.34
C22 :0	Behenic A.	0.007

The TPC extraction method was suggested by Owen and Johns[21]. 2 g of sample was added to 20 mL of Ethanol (100%) and pure distilled water for 72 hours on a shaker at 150 rpm kept in a dark refrigerator. Further, the supernatant was collected and evaporated by keeping it on water at 45°C using rotary evaporator (LABOROTA 4000 HEIDOLPH, Germany). The dry extracts were then kept at 4°C until use [21]. The obtained extracts were subsequently used for the determination of TPC and total flavonoids.

- The TPC were quantified by spectrophotometry using the Folin-Ciocalteu method [22]. The absorbance at 710 nm of both extracts (ethanolic and aqueous) was measured by a spectrophotometer (EV 9200, Germany). The regression equation of calibration was obtained with various concentrations of the Gallic Acid standard. Three replicates per treatment were employed to calculate the TPC value, expressed as mg of Gallic Acid Equivalent per g of dry basis (mg GAE/g db);
- The method suggested by Bahorun *et al.* [23] was used for the determination of flavonoids. The flavonoids were analyzed by a colorimetric method at 430 nm, and then quantified using the regression equation of calibration, which was obtained with various concentrations of the Quercetin standard and expressed by mg of Quercetin Equivalent per g of dry basis (mg QE/g db). Three replicates per treatment were utilized to estimate the flavonoids value;
- For the determination of soluble-water polysaccharides, the sample was first treated with petroleum ether (99%) under reflux of Soxhlet at 210°C for 3 hours [24] to eliminate the pigments. The obtained marc after filtration was subjected to an extraction in distilled water (80°C/2h). Further, the supernatant was treated with three volumes of isopropanol (99.5%) for 24 hours kept in a dark refrigerator in order to precipitate the polysaccharides. After filtration, the mass of the crude water-soluble polysaccharides was washed five times with acetone [25] and then dried at 40°C. Finally, the mass yield was determined;
- The tannins were extracted by precipitation using two solvents (methanol and diethyl ether) as described by Biaye [26] and Bruneton [27]. The tannin extraction yield was then calculated using the formula described by Falleh *et al.* [28].

$$\text{Tannin yield (\%)} = \frac{W1}{W0} \times 100$$

W1: represents the weight of dried extracted tannins, and W0 represents the weight of sample used.

The tannin content was determined by casein colorimetric method at 750nm [29], and then quantified by the difference between TPC content and TPC obtained after tannin complexation by casein.

- IR spectrometry (BRUKE ALPHA, China) was used to analyze the functional groupings of the crude extract to confirm the presence of hydro-soluble polysaccharides.

2.3.2 Antimicrobial activities

The sensitivity of four strains (*Escherichia coli* ATCC 25923, *Staphyloccocus aureus* ATCC 25322, *Aspergillus niger* and *Candida albicans*) with regard to the aqueous and ethanolic extract of total phenolic compounds (TPC), water-soluble polysaccharides, and tannins extracts of Muscat of Alexandria leaves were evaluated according to the method described by Ponce *et al.* [30].

Muller Hinton (MHA, Oxoid)) agar was used for the bacterial strains and Sabouraud agar was used for the fungal strains.

Extract was tested by agar disc diffusion method [31]. The medium, previously melted at 100°C and cooled to 45°C, was poured into Petri dishes and inoculated with the different strains. A suspension containing 107 UFC/mL of bacteria and 104 CFU/mL of fungal strains was spread on Mueller-Hinton Agar, or Sabouraud Agar. Sterile cellulose discs impregnated with the 10 µl extracts were

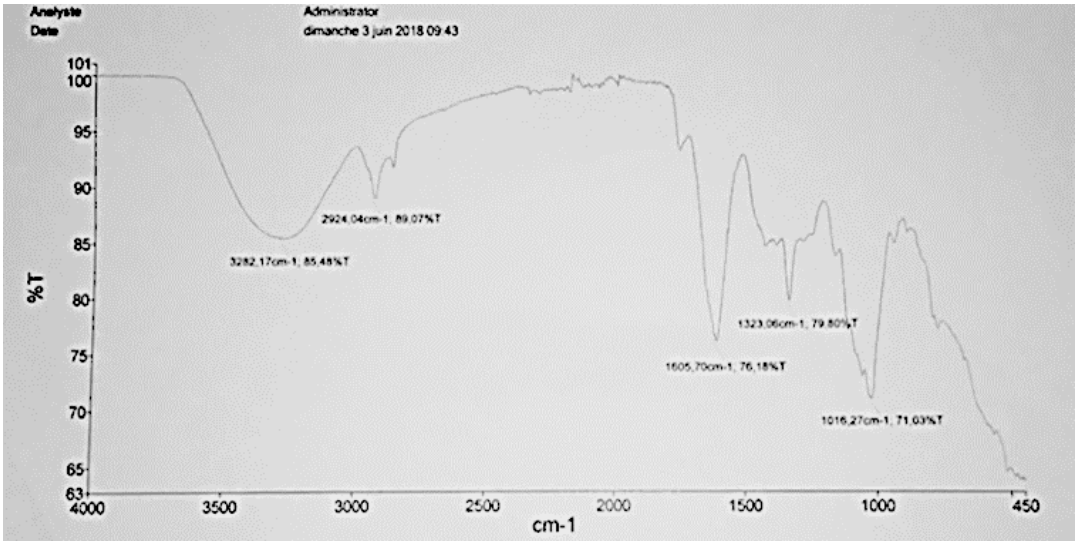

Figure 2: IR spectrum of water-soluble polysaccharides extracted from Muscat of Alexandria leaves

then placed on the surface of the agar and the plates incubated at 37°C. After 24 h of incubation the diameters of the inhibition zones were measured.

2.3.3 Preparation of fermented milk supplemented with vine leave extracts

The extract and the powder of the vine leaves were tested for their prebiotic potential on the two strains used for yogurt. The microbiological quality of the extract and the vine powder was confirmed before their use in the yogurt.

The vine leaves extracts were obtained according to the method defined by Aquilanti *et al.* [32]. Two (2) g of vine leaves powder were macerated in 100 mL of distilled water at 45°C for six hours and then filtered through a filter paper (Whatman N°4). The obtained extract was kept at 4°C.

Standard semi skimmed milk (1.5% Lipid Matter, pH=6.43±0.057) was sterilized (121°C for 3S) and kept at 45°C for 15 min before use in accordance with the method suggested by De Souza Oliveira *et al.* [33]. The powder and extract were added at different concentration to pre-warmed (45°C) milk, and the mixtures were inoculated with a commercial yogurt starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus cultures* (Chr. Hansen, Horsholm, Denmark). The inoculum was prepared using direct vat set cultures at concentration of 0.002 g of starter culture (each per liter). The mixtures were incubated at 45°C until the acidity reached 70°D (pH 4.5). The yogurt without added plant extracts was used as control in this study.

3 Results

Vine leaves present low water content (10.74%), an acid pH (4.7) and a relatively strong titratable acidity (11.84 ± 0.15%). On the other hand, total sugars, reducing sugars and sucrose are present at very low levels (Table 1).

In terms of mineral composition, the most abundant mineral compounds are Na (5039.53 mg/kg db), and K (1714.33 mg/kg db) whereas heavy metals such as (Cr, Cd) are absent.

The quantitative analysis of phenolic compounds (TPC and flavonoids) shows that their ethanolic extracts have higher levels, respectively (17.57 mg EAG/g db, 12.55 mg E Quercetin/g db) in comparison with aqueous extracts (16.27 mg EAG/g db, 10.23 mg E Quercetin/g db). Tannins are also present with a yield of 7%. These compounds are known for their anti-diarrheal properties; they reduce the permeability of the intestinal mucosa. They can also act as antiseptic agents in the case of pulmonary infections thanks to an inhibitory action on the growth of bacteria, fungi and viruses [34, 35].

It was also observed that the mass yield of the water-soluble polysaccharides of vine leaves is very low (2.66 %) compared to other results using the same extraction procedure (decoction, maceration) and the same type and amount of solvent etc. [36, 37]. Furthermore, since polysaccharides are primary metabolites, they are used as precursors for other secondary metabolites, as a source of energy.

Infrared analysis (Figure 2) of the produced water-soluble polysaccharides reveals the presence of an intense broadband at 3282 cm-1 attributed to the hydroxyl group elongation vibration (-OH), characteristic of polysaccharides, which can involve a very high reconstitution capacity (HRC) [38, 39].

Low band of asymmetric C-H bond vibration is observed at 2924 cm-1 [40, 41]. Similarly, the absorption observed around 1605 cm-1 is assigned to carboxylate groups (-COO-) [42]. In this zone, a broadband corresponds more specifically to the extensional

vibration of the C=O function of the carboxylic group (COOH) of galacturonic acid (GalA) [43]. The signal observed at 1323.06 cm-1 can be considered as specific to the ester carbonyl groups of the carboxylic function of GalA [43] elongation vibration of the carboxylate group. Generally, the band observed at 1016.27cm-1, specific to the carbohydrate C-O functions such C-O-C and C-O-H bonds of the polysaccharide structures [44, 45].

Furthermore, it was observed that the vine leaf powder contains four dominant fatty acids (Table 2): palmitic acid (26.81%), linolenic acid (12.03%), oleic acid (6.45%), and linoleic acid (5.93%). These acids can have a significant impact on human and animal health, particularly in preventing a large number of pathologies (neurodegenerative, metabolic, cardiovascular, and inflammatory diseases [46].

The antimicrobial activity (Table 3) revealed that tannins extract have better inhibition zones against *E.coli* ATCC 25923, S. aureus ATCC 25322 and *C. albicans* except *A. niger* with inhibition diameters of 30.667 ± 3.055 mm, 33.5 ± 2.121 mm and 17.5 ± 3.535 mm, respectively. With respect to TPC and polysaccharides extracts, they have an important inhibition effect against *A. niger*.

Benahmed Djilali *et al.* [5] using a mixture of *Z. jujuba* and Spirulina extracts as prebiotics and lactic leavens with a clotting speed of 130 min.

4 Discussion

It is worth noting that sufficient evidence has been accumulated in this research to show that the Muscat of Alexandria leaf powder has a high biological value that may be explained by its richness in bioactive substances (TPC, polyphenolic compounds, water-soluble polysaccharides, and fatty acids). This richness in these compounds confers to the powder important bioactive properties, which explains their use in traditional medicine and meals.

Phenolic compounds (flavonoids and tannins) have also been reported to have beneficial antioxidant [47], antimicrobial and insecticidal effects [48]. On the other hand, Muscat of Alexandria leaves is devoid of fatty acids, in particular linolenic acid, which is considered an active antibiotic [49].

The availability of these substances depends on a number of factors: the eco-physiological state (harvest period), cultivation conditions, the climatic conditions, the experimental conditions (temperature, amount of solvent) [50, 51], and the extraction method[52].

Table 3: Inhibition diameters of (TPC, polysaccharides, and tannins) extracts of Muscat of Alexandria leaves (n=3)

	TPC extracts		TPC extracts		Tannins
	AE	AE	AE	EE	
***Escherichia coli* ATCC25923**	Abs	19 ± 1.41	Abs	Abs	30.67 ± 3.05
***Staphylococcus aureus* ATCC 5322**	Abs	18.5 ± 0.70	Abs	Abs	33.5 ± 2.12
Candida albicans	Abs	18.5 ± 0.70	Abs	22 ± 1.414	17.5 ± 3.53
Aspergillus niger	47.5 ± 3.53	24.5 ± 7.77	72.5 ±17.67	51 ± 36.77	Abs

AE: Aqueous Extract, EE: Ethanolic Extract, TPC: Total Phenolic Compounds

Moreover, ethanolic extract of polysaccharides were found to possess the highest inhibition zones only against *C. albicans* and *A. niger* in comparison with ethanolic extract of TPC. We can consider both TPC and polysaccharides extracts of vine leaves as antifungal agents.

The combination of vine leaf powder and their extracts as prebiotics with lactic bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) exhibited an interesting prebiotic effect. In fact, both powder and extract significantly accelerated yogurt acidification and reduced clotting time. The fermentation was more rapidly completed in the plant extract-supplemented samples than in the control. The use of 0.5 g of vine leaf powder caused coagulation after 120 min, while the addition of 1 and 1.5 g in 50 mL of milk reduced the coagulation time by 50%. The addition of the aqueous extract caused an instantaneous coagulation. The addition of the mixture of lactic acid bacteria with vine powder provoked a reduction in coagulation time of 70 to 85% compared to the use of lactic acid bacteria alone. This speed remains very low compared to those obtained by

Vine leaf powder contains low yield of water-soluble polysaccharides in comparison to those obtained from *P. major* leaves, which had a higher yield mass of 36% and 26% using an extraction temperature of 50°C and 100°C, respectively as reported by Samuelsen *et al.* [52].

The lower amount of water-soluble polysaccharides produced can certainly be explained by the grain size, which is about 498.474µm in our case (Figure 1). Moreover, it is worth noting that the level of polysaccharides is more sensitive to the drying process than to the type of solvent used; Ginzberg *et al.* [53] suggested the drying by freeze of polysaccharides for retention of their functionality. However, low amount of sugars was observed during the harvest period. Indeed, the leaves synthesize the sugars concentrated in the grape at the end of the harvest season [54]. The difference in the biological activity of vine leaves extracts is mainly due to the difference in the chemical structure. In addition, the antimicrobial activity varied depending on the molecular weight and concentration of the oligosaccharides and type of microorganism[55].

In this work, we studied the effects of non-digestible polysaccharide prebiotic on probiotics (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) from vine leaves on yogurt fermentation.

In agreement with the results for acidification kinetics, both yogurts supplemented with vine leave extracts and powder showed a more rapid increase than the control yogurt.

The increase of the fermentation speed confirmed by an acceleration of the acidification of the yogurt in the presence of the powder or the aqueous extract of the vine confirms a beneficial effect on the growth of the lactic bacteria of the yogurt, which can suggest a prebiotic effect. However, it is certain that this effect can only be confirmed after studying the evolution of these compounds after their transit through the stomach and the gut and their resistance to the action of digestive acids and enzymes.

Different studies have reported that commercial prebiotics such as inulin, maltodextrin, oligofructose, and polydextrose accelerate the acidification and reduce the fermentation time of the yogurt[56]. The prebiotics also improve the metabolic activity of yogurt bacteria and decrease the pH due to production of organic acids. Amirdivani & Baba [57] reported accelerated fermentation related to the prebiotic effect.

Comparison between the various clotting agents in terms of pH shows that the combination promoted a decrease of pH. This decrease can be attributed to the limited post-acidification ability of the probiotic used and storage temperature [18, 58]. Indeed, it is well known that the pH decrease could be explained by the fact that *S. thermophilus* produces little amounts of formic acid and volume). This reduction directly correlates with the composition of vine leaves (fiber, polysaccharides, tannins), which involves a very High Reconstitution Capacity (HRC). However, the combination of prebiotic (powder or extract of vine leaves) with lactic bacteria *S. thermophilus* with *L. bulgaricus* are known to influence the fermented milk textural properties (firm texture) [31].

As a result, the combination of both vine leaf powder and their extract as prebiotics with probiotics exhibited an interesting clotting milk speed (Table 4). We can conclude that the mixture can stimulate milk fermentation, the metabolic activities of probiotic bacteria and the development of acidity. Similar findings were reported for ice cream supplemented with carob extract and whey powder by Guler-Akin *et al.* [15]. Indeed, their results showed that the addition of carob extract and whey powder significantly affected the viable probiotic bacteria during ice-cream freezing.

5 Conclusions

Vine leaves have several phytochemicals and nutraceuticals compounds such as polyphenols, flavonoids, tannins and polysaccharides. These compounds have interesting antimicrobial activity. The latter promotes lactic bacteria fermentation. We can use Vine leaf powder and their extract as prebiotics to improve milk clotting. As results of this fermentation, there is release of phenolic acids responsible of antimicrobial activity and stability of fermented milk.

The vine leaf can be used as a prebiotic to prepare functional yogurts that have the effect of reducing the intestinal flora responsible for many diseases.

Table 4: Some parameters of sour milk using various clotting agents (n =3)

Trial n°	Nature of clotting agents		Quantity of milk (mL)	Milk clotting time (min)	Syneresis volume (mL)	pH at 20°C
1	Lactic bacteria (mL)	1	10	103±0.006	5.83±0.05	
2	Vine leaf powder (g)	0.5	10	120±	Abs	5.4±0.007
		1	10	60±0.001	Abs	4.6±0.001
		1.5	10	60±0.02	Abs	4.6±0.05
3	Vine leaves extracts (mL)	5	5	-	Abs	5.4±0.023
		2.5	7.5	-	Abs	6±0.001
		7.5	2.5	-	Abs	5.7±0.02
4	Mixture of vine leaf powder (g) add lactic bacteria (10% V/V)(mL)	0.5	10	30±0.001	3	5.4±0.005
		1	10	15±0.002	1	4.6±0.01
		1.5	10	30±0.001	2	4.6±0.045
5	Mixture of vine leaves extracts add lactic bacteria (V/V mL)	4:1	5	2±0.005	0	4.7± 0.004
		1.5:1	7.5	2±0.002	3	5.9±0.03
		6.5:1	2.5	2±0.001	6	3.6 ±0.05

AE: Aqueous Extract, EE: Ethanolic Extract, TPC: Total Phenolic Compounds

CO_2 [59]. The obtained results in this research agree well with those reported by Seleet *et al.* [60] Benahmed-Djilali *et al.* [5], and Lukova *et al.* [16].

It was observed that whatever the nature of the prebiotic (powder or extract) of vine leaves, it had no effect on syneresis (lack

All the results obtained in this study are only a first step towards recovery of the studied byproducts. Further accurate and thorough studies are still needed.

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ORCID: Adiba BENAHMED DJILALI: https://orcid.org/0000-0001-8745-8062
ORCID: Abdelouahab BENSEDDIK: https://orcid.org/0000-0003-3823-4323
ORCID: Halima BOUGHELLOUT: https://orcid.org/0000-0002-1240-2133
ORCID: Karim ALLAF: https://orcid.org/0000-0002-7600-5903
ORCID: Mohamed NABIEV: https://orcid.org/0000-0002-9307-5276

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