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In Vitro Antioxidant Properties of *Lactobacillus gasseri* Isolated from Fermented Milk: Isolation and Preparation of Strains and Intracellular Cell-Free Extracts, Chemical Testing (Hydrogen Peroxide, Superoxide, Hydroxyl Radical, DPPH, Ferrous Ion Chelating, and Linoleic Acid Peroxidation)

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ABSTRACT

Among the most accepted hypotheses that can explain the causes of aging, we found the theory of free radicals in Lactobacillus gasseri strains. We did screen the antioxidative properties of Lactobacillus gasseri strains isolated from fermented milk. The results showed the most resistant strains against hydrogen peroxide with excellent superoxide anion radicals scavenging capacity. The results were also supported by DPPH free radical scavenging ability. We also found the greatest ferrous chelation capacity (reaching 88%). The tested strains demonstrated anti-lipid peroxidation levels ranging between 30 and 66%. The results suggest that L.gasseri could be used as an effective antioxidant to fight against diseases related to oxidative stress.

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1. INTRODUCTION

Have aged is a complicated biological process, characterized by degenerative and physiological or biochemical functional deterioration of cellular tissues. Among the most evaluated and accepted hypotheses that can explain the causes of the aging phenomenon, is the radical theory of aging (Muller *et al*., 2007). This theory aimed to examine the correlation between oxidative damage, longevity, and age-related health problems. Considering the theory of aging of free oxygen radicals, the imbalance between the production of reactive oxygen species (ROS) such as the hydroxyl radical as well as the superoxide anion and the antioxidant molecules increases in favor of oxidants (Weidinger & Kozlov, 2015). Oxidative stress is considered one of the factors responsible for the evolution of the aging process and several serious chronic health problems, mainly diseases related to the dysfunction of the immune system (autoimmune), carcinogenesis, atherosclerosis, diabetes, Alzheimer's disease, and cardiovascular diseases (CVD). Oxidative stress could be actively responsible for various abnormalities. Thus, it can cause the initiation of related pains and ailments (Simioni *et al*., 2018).

Polluted water and air, ultraviolet rays, tobacco, alcohol, and many normal cellular redox processes can be linked to the induction of high concentrations of reactive oxygen species (ROS). Thus, the importance of nutraceuticals and a diet rich in antioxidants of natural origin could prevent the human body, and therefore delay the onset or evolution of serious chronic diseases (Getoff, 2007). In recent years, researchers have focused more and more on the study of functional natural antioxidants that can substitute chemical antioxidants (Li *et al*., 2013). Currently, exopolysaccharides (EPS) have attracted considerable attention as components with an antioxidant effect that can be widely used, especially those obtained from species of lactic acid bacteria (Guo *et al*., 2013a). Lactic acid bacteria (LAB) have certain probiotic properties, which have a positive effect on human health, adjusting the balance of the intestinal flora (Klaenhammer & Kullen, 1999), minimizing cholesterol levels in plasma (Corzo & Gilliland, 1999), antimicrobial activity (Vesterlund *et al*., 2004) and revitalizing the immune system. The antioxidant capacity of LAB has only recently been studied and scientifically proven, and this by eliminating free radicals and ROS (Lin *et al*., 2000; Kullisaar *et al*., 2002).

One of the most abundant and important genera of bacteria, *Lactobacillus* spp, has been able to attract a lot of attention thanks to its potential probiotic effects and its antioxidant activities. These bacterial strains are involved in the contribution of body protection against free radicals and by decreasing, the level of ROS during the process of digestion of food, and therefore can potentially lead to the progression of various diseases. In previous studies, the antioxidant activities of lactic acid strains and their beneficial characteristics on the health of the human body associated with the control of oxidative aggressions have been reported (Tang *et al*., 2017). Various representative lactic acid species, for example, the species *L. casei, L. acidophilus, L. helveticus, Bifidobacterium longum, L. fermentum*, and *L. rhamnosus* have been proposed as functional probiotics having specific antioxidant activities (Mishra *et al*., 2015). In this study, we will mainly examine the antioxidant activities of *Lactobacillus gasseri* strains as natural antioxidants isolated from traditional Moroccan fermented milk using various dosages.

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2. METHODS

2.1. Isolation of *L. Gasseri* Strains

L. gasseri strains screened in the current study are isolated from fermented milk from farms in the eastern region of Morocco. The samples were taken in sterile carriers and kept in a cooler until they were delivered to the laboratory for biochemical and microbiological analysis on the same day. The coagulated samples were diluted aseptically in a sterile broth of Tryptone salt up to 10-8.100 microliters of each dilution were cultured on medium agar Petri dishes from De Man, Rogosa, and Sharpe (MRS) and incubated at 37°C for 24 to 48 h under anaerobic conditions, using a candle extinguishing pot. The single colonies were randomly selected, and identified by the technique of Gram staining and catalase production. Colonies with Gram-positive and catalase-negative strains having the straight rod shape have been pre-identified as LAB. (Carr *et al*., 2002). The purified colonies were maintained at -28 ° C in a sterile MRS broth homogenized with 20% glycerol. The purified bacterium was identified after testing the physiological and biochemical properties according to the standard clinical laboratory methods indicated and recommended by Sharpe (Sharpe, 1979). The carbohydrate fermentation profile of the isolates was defined using the API 50 CH gallery (Biomérieux, France).

2.2. Preparation of Strains and Intracellular Cell-Free Extracts

The six isolated strains of *L.gasseri* were inoculated in a sterile MRS broth, incubated at 37°C for 24 h, and collected by centrifugation (6000 g for 10 min, 4°C). To obtain intact cells, the cell granules were washed 3 times using phosphate-buffered saline solution (PBS) and then resuspended in a PBS solution once again. The bacterial load of the cell was adjusted to correspond to approximately 10 log CFU/mL. The acellular intracellular extracts were prepared according to the protocol described by Lin & Chang, (2000). The cell pellets were subjected to washing with deionized water and followed by ultrasonication (JY92-IIN, Ultrasonic Homogenizer, Scientz, Henan, China). The disturbance by the sonication method was carried out at 5 times (1 min) intervals in the cold bath. After the cellular debris had been removed by centrifugation (8000 rpm, 10 min, 4°C.), the supernatant produced was used as an intracellular cell-free extract of *L. gasseri* strains.

2.3. In Vitro Antioxidant Screening of The Tested *L.Gasseri* Species

2.3.1. Resistance to hydrogen peroxide

The cultures resulting from an overnight incubation of *L. gasseri* were picked by centrifugation (3000 rpm, 10 min, at 4°C), then resuspended in PBS, and adjusted to a bacterial concentration of 109 CFU/mL. The isolates were inoculated at one percent (v/v) in a sterile MRS broth mixed with 1 mM of H_2O_2 and incubated at 37°C for 48 h. Then, the aliquots were removed and spread every 2 h on MRS agar plates.

2.3.2. Superoxide anion radical scavenging property

The mixture of Tris-(hydroxyméthyl)-aminométhane chlorhydrate(Tris-HCl) (150 mmol/L, pH 8.2), three mmol/l of EDTA, 1.2 mM 1,2,3- Trihydroxybenzene, and 0.5 mL of intact cells or cell-free extract was adjusted to corresponding of 3.5 mL as total volume of reaction. This solution was incubated at 25°C for 10 min, and then the absorbance was measured with spectrophotometry at OD 325 nm. The resistance percentage to superoxide anions radical (O2-) was expressed by the following formula: % scavenging effect =1-$\frac{A11-A10}{A00-A01}$. 100%. where A00 is the sample with no *L.gasseri* strain or 1,2,3- Trihydroxybenzene, A01 is the sample with

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no *L.gasseri* but has 1,2,3- Trihydroxybenzene, A10 is the sample containing *L.gasseri* strain but no 1,2,3- Trihydroxybenzene, and A11 is the sample with contain *L.gasseri* strain and 1,2,3- Trihydroxybenzene.

2.3.3. Hydroxyl radical (.OH) scavenging capacity

The hydroxyl radical (OH*) scavenging assay was carried out according to a Fenton reaction method (Wang *et al*., 2009). The generation of OH^- was conducted in a reaction mixture containing 0.5 mL of $FeSO_4$ (8 mmol/L), 0.5 mL of sterile distilled water, and 0.8 mL of hydrogen peroxide (6 mmol/L). 1 mL of intracellular cell-free extract and 0.2 mL sodium salicylate ($C_7H_5NaO_3$) (20 mmol/L) added to the reaction solution was then incubated at 37°C for 1 h. Hydroxyl radical scavenging capacity was defined using Scavenging activity (%) = $[1 - A1 - A2/A0]$ .100%, where A0 is the absorbance obtained from the control without the sample, A1 is the absorbance in the presence of the sample, and A2 is the absorbance value without sodium salicylate.

2.3.4. DPPH free radical scavenging ability

The 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of *L.gasseri* was measured based on, the method reported by Kao and Chen (2006), with slight modifications. In brief, 1 mL of *L.gasseri* intact cells and intracellular cell-free extract exhibiting 10 9 or 1010 CFU/ mL was mixed with 2.0 mL of freshly prepared DPPH ethanolic radical solution (0.05 mmol/L). The reaction solution was mixed regularly and incubated in the dark at 37°C, for 30 min. The control solutions included just deionized water added to a DPPH solution. The controls included only ethanol and bacterial cells. The resulting solutions were then calculated by measuring their absorbance at 517 nm after centrifugation had been carried out (8000 rpm; 10 min). The scavenging capacity was determined using (%) Scavenging activity= $\frac{[1-(Absorbance\ of\ sample-Absorbance\ blank)}{\text{Absorbance control}}$.100%.

2.3.5. Determination of ferrous ion chelating capacity

Ferrous (Fe^{2+}) ions chelating evaluation was conducted as described by Lin and Chang (2000). In short, 0.5 mL of intracellular cell-free extract previously prepared has been added to 0.1 mL of ascorbic acid (Vitamin C) (1%, weight/volume), 0.1 mL of Ferrous Sulfate (0.4 g/L), and one mL of Na OH (0.2M). The reaction mixture was incubated at 37°C in a water bath for 20 min, and then 0.2ml of Tri-Chloroacetic Acid (TCA 10%) was added to the mixture solution and followed by incubation for 20 min. Supernatant from the studied samples was collected by centrifugation at 6.000g for 10 min, and 0.5 mL of o-phenanthroline compound ($C_{12}H_8N_2$; 1 g/L) was then added. Estimation of chelating propriety was performed with spectrophotometry at OD 510 nm. The percentage of (Fe^{2+}) ions chelating was determined based on the following formula: Ferrous ions (Fe^{2+}) chelating (%) = [1-(OD1⁄(OD2)]) x100%, where OD1 is the optical density of the sample, and OD2 is the absorbance of the control. The control solution comprised the reaction medium. The sample was replaced by distilled water.

2.3.6. Measurement of linoleic acid peroxidation

Linoleic acid was selected as the source of unsaturated Fatty Acid (F.A) tested in this study (Bertelsen *et al*., 1995). The thiobarbituric acid (TBA) technique was adopted for the determination of lipid peroxidation, and the Fe/ascorbic acid system was used to catalyze the oxidation process. 30 mL of linoleic acid emulsion was formed by a homogenization of one mL of linoleic acid, 0.2 mL of Polysorbate 20 and 19.7 mL of deionized water. 0.5 mL of PBS

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(0.02 M, pH 7.4) were then added to 1 mL of linoleic acid emulsion, 0.2 mL of ferrous sulfate ($FeSO_4$) (0.01%), 0.2 mL of ascorbic acid (0.01%) and 0.8 mL of intact cellular and acellular intracellular extracts. The reaction mixture obtained was then incubated at a temperature of 37°C for 12 h. Then, 2 mL of the reaction solution was added to an equal volume of 0.2 mL of TCA (4%, weight /volume), 2 mL of tertiary butyl alcohol (TBA, 0.8%), and 0.2 mL of butylated hydroxytoluene (BHT, 0.4%, w/v). The solution produced was homogenized in a magnetic stirrer followed by incubation for 30 min at 100°C. It was then cooled to ambient temperature, and for extraction, 2 mL of chloroform ($CHCL_3$) was added. The absorbance of the solution obtained was measured spectrophotometrically at a wavelength of 532 nm (A sample). The blank containing 0.5 mL of PBS is used to replace the sample (A0). The rate of elimination of the process of peroxidation of linoleic acid (C18H30O2) was determined according to the following formula: % Elimination of peroxidation of linoleic acid = [1-A (sample)/A (blank)] × 100%.

2.4. Statistical Analysis

All statistical assessments were conducted by using ANOVA and Duncan's new multiple tests ($P < 0.05$) by using SAS version 9.4 software. The experiments of the study were performed in triplicate at three independent times (n=3) and the results were presented as mean ±Standard Deviation (SD).

3. RESULTS AND DISCUSSION

3.1. In Vitro Antioxidant Properties of *L. Gasseri* Strain

3.1.1. Resistance of intact cells to hydrogen peroxide

Relatively, hydrogen peroxide is considered a less powerful oxidizer, but it is characterized by a high diffusion and a long service life. According to these two essential characteristics, H_2O_2 contributes to the oxidative damage of cells either in a direct way or as a precursor element of hydroxyl radicals (HO) which are very reactive oxidants. The survival of each strain of *L. gasseri* was evaluated in a medium containing H_2O_2 (1 mm). The five strains were viable even after 8 h of incubation, despite differences observed in the survival rate. The strains G5 and G6 were the strains most resistant to H_2O_2, with many viable cells greater than 5 and 5.1 CFU/mL, respectively. However, 50% of the G12 had lost its viability after 2 h while the strains, so it is considered the most sensitive strain. In addition, the intact cells G2 and G11 retained their viability after the end of incubation (8 h) (see **Figure 1**).

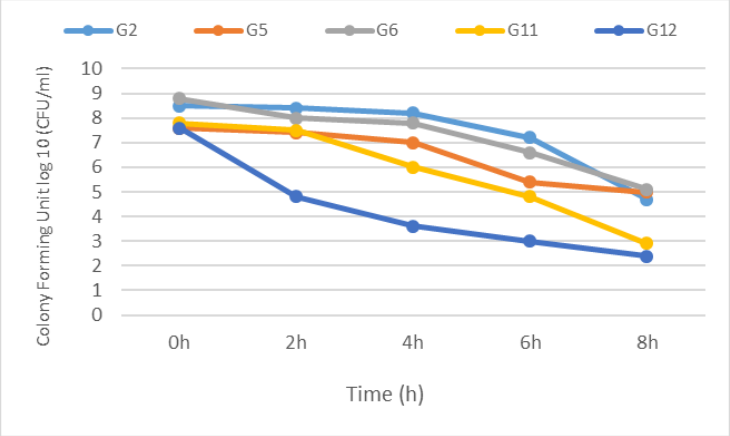

Figure 1. Intact cell viability of *L.gasseri* strains exposed to 1 mM H_2O_2 at 120-min time intervals, the rate of the viable cell was calculated by plating the removed aliquots onto MRS- agar plates**.**

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The results obtained, in which the intact cells are resistant to H_2O_2, are not shocking because among the mechanisms allowing lactic acid bacteria to succeed in their resistance in the gastrointestinal tract (GI) of a host organism is their secretion of antimicrobial metabolites such as lactic acid, bacteriocins, as well as Hydrogen Peroxide (Ouwehand & Vesterlund, 2004). The result showing the very significant survival of the G5 and G6 strains in an environment containing H_2O_2 supported the findings reported by Talwalkar & Kailasapathy (2003) who found that even after exposing the *L.* acidophilus strain to a concentration of 30,000 mg/ l of H_2O_2 for 1 min, the strain did not show a significant decline in cell viability. After 4 h of incubation with hydrogen peroxide (1 mM), the *L. fermentum* strains showed a strong survival exceeding 90% of cell viability (Wang *et al*., 2009). The lactic strains *L. casei* KCTC 3260 and *L. rhamnosus* GG preserved their viabilities after 8 h of exposure to 1 mM of H_2O_2 (Lee *et al*., 2005), when the cells of *L. fermentum* E-3 and E-18 kept their survival for only 3 and 2.5 h, respectively (Carr *et al*., 2002). The cells of *L. casei* 01 (Lee *et al*., 2005) as well as *L. fermentum* E-338-1-1 (Kullisaar *et al*., 2002), have been characterized as being sensitive to Hydrogen Peroxide. The mechanism that can explain the apparent variability of resistance to the same molecule (H_2O_2) by various Lactobacillus strains can be explained by the differences in the initial cell rate, as well as the differences in the Lactobacillus species used in the tests.

3.1.2. Superoxide anion radical (O^{2-}) scavenging

In most organisms, (O^{2-}) plays the role of its precursors of active free radicals (Rout & Banerjee, 2007). In comparison, the hydroxyl radical (OH^-) is considered a more selective agent because it has been shown to establish rapid reactions with the polypeptides of the [4Fe-4S] cluster, while the hydroxyl radical can react with proteins, lipids, and nucleic acids, inducing oxidative damage. Due to the ability to diffuse with [4Fe-4S] cluster structures, the superoxide anion can cause an increase in hydrogen peroxide levels, which rapidly attacks neighboring molecules (De Freitas & Meneghini, 2001). In the present study, superoxide anionic radicals were produced using Benzene-1,2,4-triol, which can reduce to stable cationic radicals but remain sensitive to Oxygen.

As is clear from **Figure 2,** the cell-free extract of all strains tested has proven a greater superoxide anion scavenging capacity than intact cells, where the values vary between 71% for the G6 strain and 48% for the G12 strain. According to Guo *et al*. (2013b), these figures are the result of the ability of the EPS present in the supernatant. Furthermore, the intact cells exhibited scavenging ability, which varies between 35% (G12) and 18% (G6). Duz *et al.* (2020) and Rwubuzizi *et al*. (2023) have obtained similar or more or less high values. Among the relevant mechanisms that allow cells with antioxidant activity to ensure their enzymatic protection, we find the enzyme: the superoxide dismutenzymaticase (SOD), which has been identified in the LAB (Ji *et al*., 2015).

Ji *et al*. (2015) investigated the SOD enzymatic activities in 5 *Lactobacillus* species, and indicated that the majority of these strains possessed the ability of scavenging exceeds 35%. In this study, all isolates of *L. gasseri* tested showed resistance to Superoxide Anions, when the values of scavenging superoxide anions were observed in intact cells and cell-free extract solutions. In the present work, all tested *L.gasseri* strains were considered resistant to the superoxide anions, and the values of intact cells and cell-free extract-scavenging superoxide anions were significantly different ($P<0.05$)

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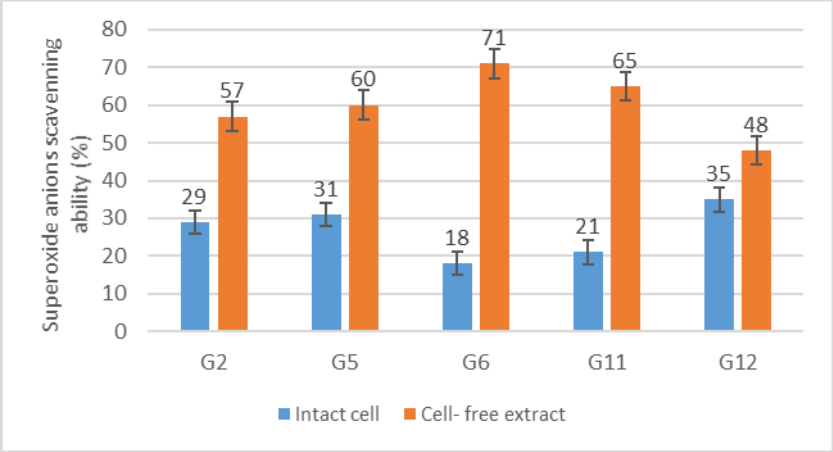

Figure 2. The scavenging ability of *L.gasseri* strain on superoxide anion radical.

3.1.3. Hydroxyl radicals scavenging ability

Hydroxyl radicals (HO*) are considered the essential agents of free radicals that attack organic molecules, thus causing serious damage to biological tissues in humans (Hazra *et al*., 2008). During our study, the generation of hydroxylated radicals was carried out by the reaction of "Fenton". The scavenging results of hydroxyl radical using the intact cells and intracellular cell-free extracts of tested bacteria are presented in **Figure 3**.

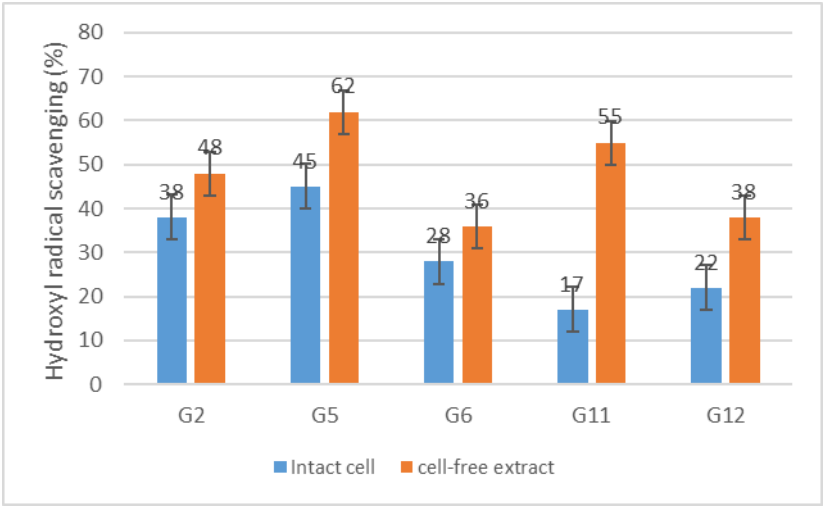

Figure 3. The scavenging capacity of *L.gasseri* strains on hydroxyl radicals.

The five strains cell cell-free extract were able to eliminate Hydroxyl Radicals (HO*). The cell-free extract of (G5) exhibited strong removal hydroxyl radical ability by 62%, much higher than that of intact cells that showed scavenging capacity on hydroxyl radical ranging from 45% for G5 and 17% for G11. Based on our findings, the cell-free extracts of G5 and G11 proved an increase that tends towards Hydroxyl Radicals. The scavenging capacity of hydroxyl radials of our *L.gasseri* strains could be explained by their possession of enzymes with intracellular antioxidant activity, in particular SOD and Glutathione Peroxidase. Although, certain non-enzymatic antioxidant metabolites, could also be synthesized within the lysates of lactic acid bacteria cells, in particular Glutathione (GSH) and Thioredoxin. In addition, the antioxidant properties of intact cells of lactic acid strains could be due to certain compounds produced at the cell surface, containing macromolecules of extracellular polysaccharides, which has been demonstrated in *Lactococcus lactis* subsp. *lactis* strains (Pan & Mei, 2010),

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Bifidobacteria (Xu *et al*., 2011). Furthermore, lipoteichoic acids (LTAs) produced on the cell surface of *Bifidobacteria* strains have proven their antioxidant effect (Yi *et al*., 2009).

3.1.4. DPPH free radicals scavenging property

The simplicity, rapidity, specificity, and repeatability, make the technique of DPPH radicals scavenging among the most adopted antioxidant tests by comparing it with other methods. The method is based on the principle that following the addition of a hydrogen donor antioxidant to the ethanol solution of DPPH, the latter is reduced, causing the development of the non-radical form (DPPH-H). The antioxidant capacity of strains raises in ratio with the reduction of the DPPH radical from purple color to yellow colored DPPH. Recorded radical scavenging activity for the studied bacteria at 1010 CFU/ml, demonstrated intact cells exhibited the highest activity 81% by G11 in comparison to that of cell-free extracts with 43% by G2 (see **Figure 4**).

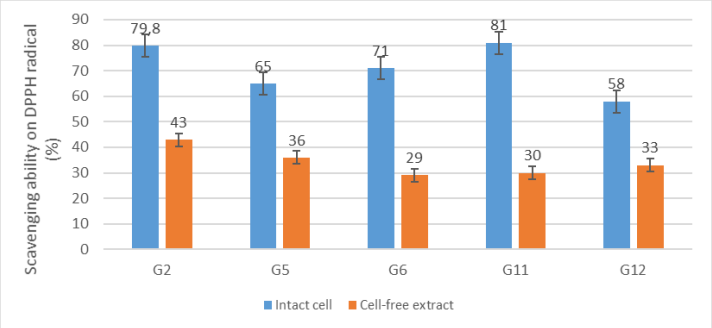

Figure 4. The scavenging ability of *L. gasseri* strains on DPPH radical.

Previous research has reported a link between the strong antioxidant property of LABs and their ability to produce extracellular Polysaccharides (Pan & Mei, 2010) or, for Bifidobacteria species, LTAs (Yi *et al*., 2009). Zhang *et al*., (2011) have well demonstrated the power of scavenging the DPPH radicals of the different species of the studied Lactobacilli, (Lacticaseibacillus *casei* subsp. *casei* (SY 13) and L.delbrueckii subsp. *bulgaricus* (LJJ)) as 23.99% and 27.50%, respectively, less than what was found in the present study. Ji *et al.* (2015) and Duz *et al.* (2020) reported for DPPH scavenging activity of Lactobacilli species, with approximate values of 50%. In the same evaluation context, and a study reported the DPPH scavenging values of *L. casei* strain (EMP2) as 78.5%, and for *L. delbrueckii* subsp. *bulgaricus* strain between 56.3% and 77.7% respectively. These findings follow our results. Moreover, Wang *et al.* (2009) revealed a level of DPPH scavenging that could be dependent on the concentrations of cells adjusted in the experimental protocol of the study. Other studies have indicated that the DPPH values could be linked to the peptide chains released because of protein degradation. (AlKalban *et al*., 2019). Besides, Talib *et al.* (2019) reported that the DPPH scavenging activity of *Lactobacillus* spp isolated from Kefir, tested in the presence of phenolic and flavonoid composts, is based on their biological properties.

3.1.5. Ferrous Ions chelating activity

In the field of the evaluation of antioxidant activity, the control of iron, in particular ferrous iron, is important because they are considered extremely reactive ions, the most abundant as well as powerful catalysts for the creation of free radicals, causing tissue damage. In addition, ferrous ions stimulate lipid peroxidation, and therefore the chelation of iron is a crucial antioxidant activity, that protects against lipid peroxidation. Therefore, the chelation capacity of Iron ions of *L.gasseri* isolates has been studied.

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As shown in **Figure 5**, the intact cells and the cell-free extract of all the strains tested, showed significant ferric ion chelation activity. The chelating activity of the intact cells was more powerful than that of the cell-free extract. The intact cells of G6 have well demonstrated their chelation capacity with very important values reaching up to 88%. Lee *et al*. (2005) reported that *L. casei* KCTC 3260 appears to be capable of chelating ferrous ions instead of increasing the concentration of SOD, thus causing the blocking of the oxidative chain reaction. The results of this study report a wide range of chelation power of Fe^{2+}. The most powerful value was 88% (G6). Compared to our results, Duz *et al*. (2020) recorded the effects of chelation of ferrous ions between 20% and 75% for all investigated strains. The chelating capacities of LAB could be due to iron chelating proteins, that are either fixed on the wall or those excreted externally, which can capture iron and inhibit its oxidation.

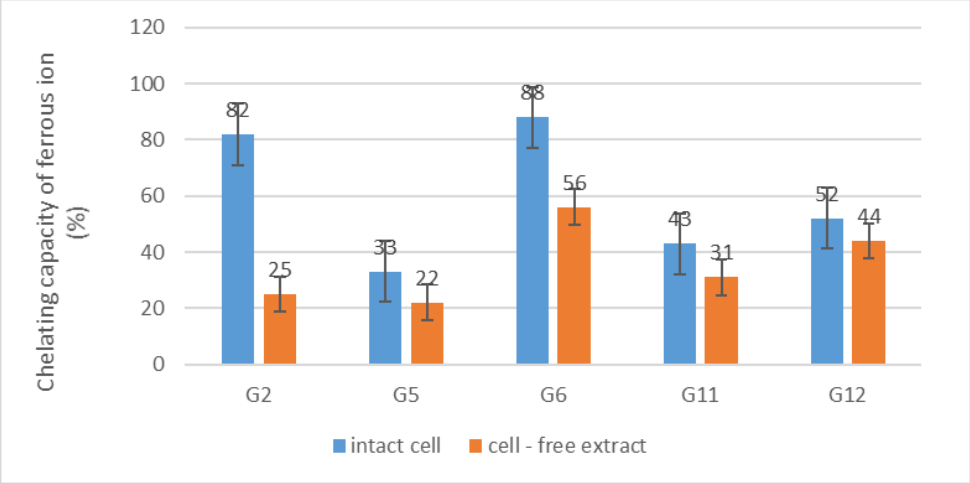

Figure 5. *L.gasseri* strains ferrous ions chelating activity.

3.1.6. Measurement of linoleic acid peroxidation

The process of oxidation of phospholipids of cell membranes in the form of peroxide derivatives is also known as Lipid Peroxidation (Yarsan *et al*., 1999). Linoleic Acid (LA) as an unsaturated fatty acid is usually most often used. As shown in **Figure 6**, the intact cells as well as the acellular extract of *L. gasseri* strains exhibited the inhibition of the peroxidation of Linoleic Acid. Based on the findings obtained in **Figure 6**, the intact cells of tested isolates demonstrated an anti-lipid peroxidation rate ranging between 44% (G11) and 66% (G6), and the cell-free extract between 30% (G2) and 49% (G12). Therefore, all the bacteria studied in this study revealed very important values of the ability to inhibit the peroxidation of linoleic acid. These results are similar to several previous researches, which were able to report the antioxidant activity of the LAB, namely the anti-peroxidation effect, such as those conducted by Lin *et al*. (2020), which demonstrated the effects of the strains *B. longum* and *L. acidophilus* on the inhibition of plasma lipids of peroxidation and obtained respectively 16.2% and 11.3%. Moreover, Zhang *et al*. (2011) reported that the intact UFC of 109 cells of SY13 *L. casei* subsp. *casei* and 108 cells of LJJ *L. delbrueckii subsp* demonstrated respectively 62.95% and 59.63% inhibition activities of the peroxidation of LA. Finally, the results obtained by Zhang *et al*. (2017) carried out on the lipid peroxidation of the strains *L. paracasei* SR10-1 and *L. curvatus* SR6 showed that the inhibition of the lipids peroxidation of *L. paracasei* SR10-1 strain (63 .89% ± 0.93%) was clearly higher than those of *L. curvatus* SR6 (55 .00% ±5. 19%). Similar results were reported by Rwubuzizi *et al*. (2023) in which the inhibition values of lipid peroxidation varied between (69.43%) and (26.15%) by the strains *L. gasseri* ST16HK and *E.faecium* ST7319ea respectively.

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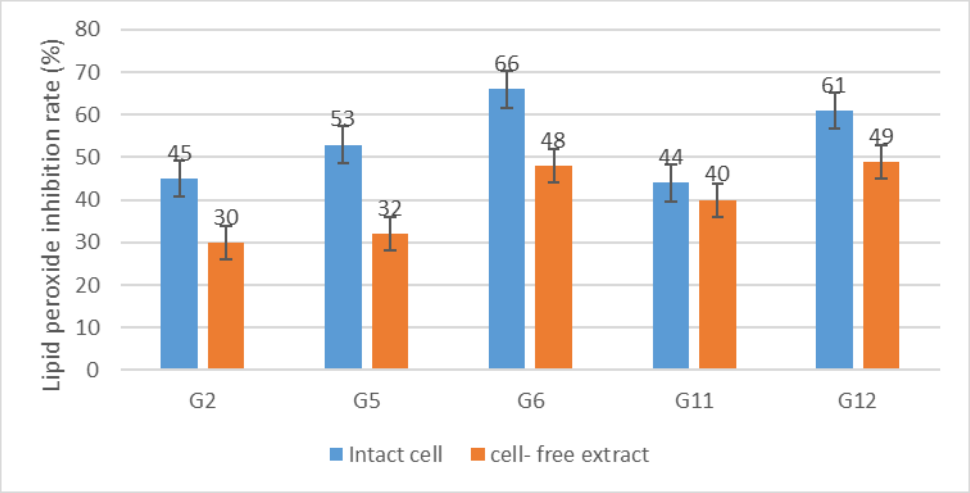

Figure 6. Inhibition activity of *L.gasseri* strains on Linoleic Acid (LA) peroxidation.

4. CONCLUSION

According to the findings obtained from the present study, *L. gasseri* strains revealed significant antioxidant properties such as hydrogen peroxide resistance ability, DPPH and Hydroxyl radicals scavenging activity, ferrous ions chelating activity, and inhibition of linoleic acid peroxidation. Hence, *L.gasseri* could be considered a highly antioxidative bacterial strain. It can extend fields of application of some Lactic Acid Bacteria as functional strains, and give realistic opportunities to decrease the application of

5. AUTHORS' NOTE

The authors state that there are no conflicts of interest in the publishing of this work. The authors certified that the data and the paper are original and free of plagiarism.

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