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# Phytochemical composition and antibacterial activity ofdate syrups extracted from Hamraya and Litime cultivars in southeastern Algeria

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## Abstract

The present study aims to develop date syrups extracted from cultivars with low market value (Litima and Hamraya), in order to contribute to the valuation of this phoenicultural patrimony firstly, and improvement of a dietary product on the other hand, by a simple technological method. The adopted method is based on the diffusion of soluble solids in water at two different temperature concentrations 65°C and 105 °C. At first, the qualitative characterization is targeted by phytochemical screening. Quantitative analysis is carried out by dosing phenolic compounds, flavonoids and condensed tannins. The biological activity is evaluated by testing prepared syrups against four bacterial strains (*Staphylococcus aureus, Echerichia Coli, Pasturellasp, Psodomonas*). The obtained results note the presence of flavonoids, tannins, coumarins, alkaloids, anthocyanins, terpenoids, cardiotonic glycosides and essential oils in the syrups of both cultivars. Polyphenols content varies between 9.19 – 13.10 mg equivalent of gallic acid/100 g of syrup, flavonoids content fluctuates between 0.94 – 2.10 mg equivalent of rutin/100g of syrup and tannins content oscillates between 0.75 – 1.57 mg equivalent of catechin/100g of syrup. However, antibacterial activity shows that *Staphyloccusaureus* germ is most sensitive against Litime105°C syrup and *Pasteurellaspp* against Hamraya65°C syrup with an inhibition zone of 10mm. Overall, the syrups produced from all cultivars combined show a significant phytochemical composition and an interesting biological activity. So, they can be integrated into the diet of the local population because of these therapeutic properties.

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## Introduction

Oasis agriculture, which is practiced intensively in a desert environment or strongly marked by aridity, is based on the cultivation of the date palm (*Phoenix dactylifera* L.), to which are associated other crops: vegetable, arboreal or fodder to form what is called the oasis ecosystem (Chniti, 2015). Algeria has an important phœnicicole potential with a thousand cultivars inventoried, the Algerian date production is substantial. Algeria is ranked the fifth in the world in date production with about 710 000 tons (Belkacemiand Rahmani, 2019). The date palm is considered the tree of the desert regions of the globe known for their hot and dry weather. Due to its dietary, ecological, social and economic virtues, the date palm is the most appreciated fruit tree by the populations of the oases (Trichine, 2010). These fruits (dates) are very numerous, only a few have commercial importance and others have a low market value. Dates are very rich in essential nutrients including amino acids, antioxidants and some mineral elements (Chafiet*al.*, 2015). Dates pounded in water cure hemorrhoids, constipation and also jaundice. As for diarrhea, it is treated with toning green dates. Or it is used as a sedative in the form of very concentrated syrup, the robb, this preparation soothes and puts children to sleep. It is also used for nervous diseases and in bronchopulmonary diseases.

In decoction or infusion, dates treat colds (Ben abbas, 2011). Despite these therapeutic virtues, many date cultivars remain poorly exploited or even marginalized. In order to contribute to the safeguarding of the phoenicicolepatrimony (more than 1000 cultivars) threatened by genetic erosion, it is important to find serious outlets for this fruit, through trials to develop new nutritional and dietary products, including a date syrup by technological means. Date syrup is greatly recommended for children, convalescents, pregnant women...), it confers important properties in terms of nutrient supply. In terms of dietary, this syrup is prepared from dates which contain secondary metabolites called phenolic compounds. These have anti-inflammatory, antioxidant effects, lower blood pressure and reinforce the immune system (Ben abbas, 2011).

This work focuses on the preparation and characterization of date syrups prepared from two date cultivars at high risk of genetic erosion. These cultivars, Hamrayaand Litime, are rarely implanted or identified by the local population due to their low market value. The date syrups' commercialization would definitely increase the revenue and utility of these dates and encourage maintenance of these cultivars by local farmers. The work also investigates the composition and properties of these syrups in regards to phytochemical groups as well as their antibacterial activity.

## Material and methods

### *Material*

The selection of Hamraya(Ha) and Litima (Li) cultivars is justified by their relative abundance on the national territory (South-East region of Algeria), their low market value, their taste quality, their classification among cultivars subject to genetic erosion and their nutritional value (energy source). These two cultivars are harvested in September 2020 at the stage of full ripening (semi-soft consistency) (Fig.1). In general, these cultivars can be used for immediate consumption or intended for technological transformation into several products including vinegar, syrup ... etc.Bacterial strains are used to evaluate the antibacterial activity of different date syrups prepared from Hamraya and Litimacultivars, we have acquired four (04) bacterial strains (*Staphylococcus aureus, Echerichia Coli, Pasturellasp, Psodomonas*) from the central laboratory of Mohamed Boudiaf hospital of Ouargla, in May 2021. These strains are preserved in petri dishes inside solid medium (Muller Hinten) at a temperature of 4 ° C.

## Methods

### *Preparation of syrups*

The extraction method adopted in this present study is based on simple diffusion laws. This method allows the passage by passive transport,fromsoluble solids

material of the plant cells of the dates into the solution (hot water or juice) through the cellulosic membrane(Albertset*al.*, 2002).It is done hot, at 80 ° C for 3 hours by addition of three volumes of distilled water at a rate of one weight of dates by three volumes of water (w / v) (Kg / l). The concentration of the juice is carried out by evaporation of free water, at 65 ° C and 105 ° C. The purpose of evaporation is to obtain saturated syrup with a Brix degree included between 72 - 74 °Brix.

*Qualitative analysis of date syrups*

Qualitative analysis allowsto highlight the presence of some chemical compounds transferred from dates. The analysis is carried out by phytochemical tests of the colored reactions (Phytochemical Screening). The phytochemical groups tested by Screening are alkaloids, flavonoids, anthocyanins, tannins, saponins, steroids, coumarins, sterols, terpenes, cardiotonicglycosides, essential oils and reducing sugars. The concept of these tests is based either on the formation of insoluble complexes using precipitation reactions, or on the formation of colored complexes using staining reactions.

*Dosing of phytochemical compounds*

Dosing phenolic compounds is carried out with presence of Folin-Ciocalteu reagent. This reagent is a mixture of complexes of phosphotungsten acids and phosphomolybdenum with yellow color. The principle of this method is based on the oxidation of phenolic compounds by this reagent, it leads to the formation of a new molybdenum-tungsten complex with blue color that absorbs at 760 nm, using spectrophotometer (UV-visible model DR 5000 HACH LANGE). The concentration of phenolic compounds is determined from a gallic acid calibration curve and expressed by milligrams equivalent of gallic acid /100g of date syrup (Laouini, 2014). Dosing flavonoids is based on the formation of a yellow complex between aluminum chloride and oxygen atoms present on carbons 4 and 5 of flavonoids, the concentration is determined using a rutin calibration curve, the absorbance is read at 410 nm (Gourchala, 2015). Dosing condensed tannins is carried out by the butanol-HCl method. The method is based on the transformation of proanthocyanidins into anthocyanidins by the rupture of interflavinic bonds in acidic medium (HCl) followed by oxidation in presence of $Fe^{+3}$ (Gourchala, 2015). The results are expressed in milligrams of catechin /100g of date syrup.This analysis was supported by ANOVA (analysis of variance) statistical analysis by Tukey's model.

*Antibacterial activity*

The antibacterial activity of date syrups is carried out by the disc method. The strains are distributed in a nutritious broth for later uses (from 18 h to 24 h after transplanting). The culture medium used is Müller Hinton.The medium is melted in a steamer at 95 ° C then poured into petri dishes of90 mm diameter at a rate of 15 ml per box. Date syrups are dissolved in dimethyl sulfoxide (DMSO) in a concentration of 1 mg/ml. The seeding of isolated bacterial strains by three pure colonies is done by flooding so as to cover the agar surface. Seeded boxes are dried at 35 °C for 15 minutes (Daasamiour *et al.*, 2014). An extract is considered active if the diameter of the inhibition zone is greater than 7 mm.

**Results and discussion**

*Date syrups properties*

The color of the syrup depends on the extraction treatment used, the date color and the storage duration. The date syrups used in this study are obtained by the diffusion extraction method. They have a clear appearance at two temperatures (65 ° C and 105 ° C), they don't need clarification processes. These syrups have taken the color of dates from which they derive namely : the syrup resulting from Hamraya cultivar has a dark red color, while that resulting from Litima cultivar has orange color, the temperature of extraction of concentration seems not to have affected the color of the syrups. (Fig. 2 A ; B).The storage duration of date syrups could cause the change in their color in other words can promote non-enzymatic browning (Maillard reaction) which causes the development of melanoidins.

**Table 1.** Photochemical screening of date syrups of Hamraya and Litima cultivars.

| | Syrups date of Hamraya cultivar | | Syrups date ofLitimacultivar | |
|---|---|---|---|---|
| Secondary metabolite | G 65°C | G 105°C | T 65°C | T 105°C |
| flavonoids | + | + | + | + |
| tannins | + | + | + | + |
| coumarins | + | + | + | + |
| anthocyanins | + | + | + | + |
| alkaloids | + | + | + | + |
| terpenoids | + | + | + | + |
| Saponosides | + | + | + | + |
| cardiotonic glycosides | + | + | + | + |
| steroids | - | - | - | - |
| sterols | - | - | - | - |
| anthraquinones | - | - | - | - |
| essential oils | + | + | + | + |

+ Presence;- Absence.

Concentration or dehydration is a physical property of water in a food. Technological treatments such as heating, environmental changes cause variations in water content of foods (Alaisand Linden, 1987). However, the biological activity of water (aw) is essential in food because it makes possible to realize a food protection strategy by controlling physico-chemical deterioration, enzymatic activities and the multiplication of microbial populations (Alaisand Linden, 1987). Condensation remains an essential operation to reduce the development of microorganisms, inhibit enzymatic reactions and facilitate storage and therefore the preservation of the product (low aw) (Chefteland Cheftel, 1984). In the present study by the concentration of syrups at 65 ° C and 105 ° C, the rate of soluble solids was optimized at 72 ° Brix in order to obtain syrups with low water activity.

**Table 2.** Variation in the content of phenolic compounds, flavonoids and tannins in date syrups depending on the cultivars.

| Sample<br>Concentration temperature (° C) | | phenolic compounds | flavonoid | condensed tannin |
|---|---|---|---|---|
| 65 | Hamraya | 11,54 (a) | 00,94 (a) | 01,45 (a) |
| | Litime | 13,10 (a) | 02,10 (b) | 01,02 (a) |
| 105 | Hamraya | 9,20 (a) | 1,19 (a) | 0,75 (a) |
| | Litime | 9,46 (a) | 1,65 (a) | 1,58 (b) |

The extraction yields of date syrups at 105 ° C and 65 ° C are comparable; namely Ha 105, Ha 65 and Li 65 is equal to 12.5%,13.5% and 12.5% respectively, except that obtained by Li 105 (8.5%) seems slightly low compared to those previous (Fig. 3).

The extraction yield of date syrup is directly related to the extraction method used and the consistency of the date. Extraction yields by the traditional process (settlement) varies between 10 and 15% (Siboukeur, 1997). Madani and Seddiki (2019) reported an extraction yields with a double diffusion significant for dates of soft cultivars Ghars and Litima (46 - 66.5%), compared to those recorded for half-soft dates Tafezwin et Takarmus (57- 56.31). Low yield on date syrups would probably due to the low water content of the opposing date, or even prevents the diffusion of sugars. Dry or semi-soft dates require enough long time to moisten and allow soluble solids to diffuse into water (Mimouni, 2015).

**Table 3.** Antibacterial activity (inhibition zones in mm) of Hamraya date syrups (Ha).

| Concentration mg/ml | Syrups Ha | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| bacterial strains | Ha 65°C | | | | | | Ha 105°C | | | | | |
| | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 |
| *Echerichia Coli* | - | - | - | - | - | 3 | - | - | - | - | - | - |
| *Staphylococcus aureus* | - | - | - | - | - | 5 | - | - | - | - | - | 2 |
| *Pseudomonas* | - | - | - | - | - | 7 | - | - | - | - | - | 4 |
| *Pasturellaspp* | - | - | - | | - | 9 | - | - | - | - | - | 7 |

- : resistant strain.

*Qualitative characterization*

The results of the phytochemical tests are listed in table 1. According to this table, we note the presence of flavonoids, tannins, coumarins, alkaloids, anthocyanins, terpenoids, cardiotonic glycosides, Saponosides, essential oils and absence of sterols, steroids, anthraquinones in the syrups of Hamraya and Litima cultivars. Phytochemical screening of the secondary metabolites required, shows that the results of all date syrups produced at two concentration temperatures (65 ° C and 105 ° C) are comparable, this leads us to say that the concentration temperature did not influence the phytochemical quality of date syrups.

**Table 4.** Antibacterial activity (inhibition zones in mm) of Litima date syrups (Li).

| Concentration mg/ml | Sirop Li | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| bacterial strains | Li 65°C | | | | | | Li105°C | | | | | |
| | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 |
| *Echerichia Coli* | - | - | - | - | - | - | - | - | - | - | | - |
| *Staphylococcus aureus* | - | - | - | - | - | - | - | - | - | - | - | 10 |
| *Pseudomonas* | - | - | - | - | - | - | - | - | - | - | - | - |
| *Pasturellaspp* | - | - | - | | - | - | - | - | - | - | - | 7 |

- : resistant strain.

*Phenolic compounds*

Polyphenol content of date syrups of studied cultivars is equal to 11.54, 9.19, 13.10 and 9.38 mg equivalent of gallic acid /100g of date syrup for Ha 65°C, Ha105°C, Li 65°C and Li105°C respectively (Table 2). Statistical analysis of ANOVA by Tukey's test did not show any significant difference between cultivars. However, the temperature variable showed a significant difference in the same cultivar (Fig. 3).The obtained values at 65 ° C for syrups of the two cultivars are high compared to those found at 105 ° C for syrups of the two cultivars, this may be due to the effect of the high concentration temperature, which probably affects the phenolic composition (modification) . According to Saci and Tliba (2019) the polyphenol content for the Cultivars Tamjouhert and Takermoust is equal to 6.94 and 4.84 mg equivalent of gallic acid /100g respectively. These values appear to be low compared to those obtained in the present study, this may be justified by the effect of the extraction temperature which could transfer significant amounts of soluble solids. Significant contents of polyphenol found in different cultivars show that dates are a considerable source of natural antioxidant and could be considered as functional food (Al-Farsi and *al.*, 2005).

The flavonoid contents obtained for the date syrups of two studied cultivars are equal to 0.94, 1.18, 2.10 and 1.65 mg equivalent of rutin /100g for Ha 65°C, Ha105°C, Li65°C and Li105°C respectively (Table 2). Statistical analysis showed a significant difference between cultivars at 65 ° C.


**Fig. 1.** Cultivars: A ; Hamraya, B ; Litima.

In addition, the temperature variable a shows a significant difference for the cultivar Litime (Fig. 3).Similarly, the value obtained at 65 ° C for the syrup of Litima cultivar is high compared to that found at 105 ° C and the opposite is reported for the syrup of Hamraya cultivar, this may probably be due to the variability between the cultivars and / or to the high temperature of concentration that could affect the phytochemical composition of the date syrups. Sayah, (2018) noted flavonoid contents of the Ghars cultivar at Routab stage around 0.20 mg equivalent of rutin /100g of dates. Ben Abbas, (2011) evoke flavonoid contents of the fresh material fluctuate between 0.33 and 0.66 mg equivalent of rutin /100g. We note that the maturation stage influences the flavonoid content. Overall, the flavonoid content of the dates cited in the literature or that mentioned in the present study remains significant.

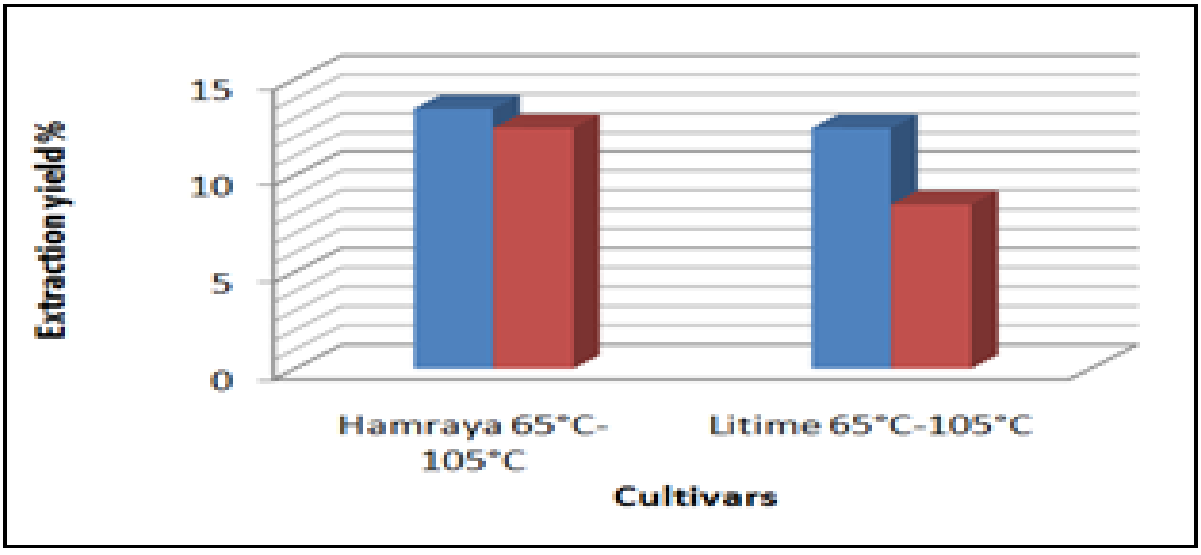


**Fig. 2.** Date syrup yield of studied cultivars.

The same remark was observed for tannins;a significant difference was recorded between cultivars at 105 ° C.Similarly, the temperature variable showed a significant difference for the cultivar Litime (Fig. 3). In general, the difference in the contents of phytochemical compounds can be explained by the influence of certain factors namely: date cultivar, maturity, storage conditions, fertilizer use, soil type, season, geographical origin and quantity of light received (Al-farsi and *al.*, 2008).

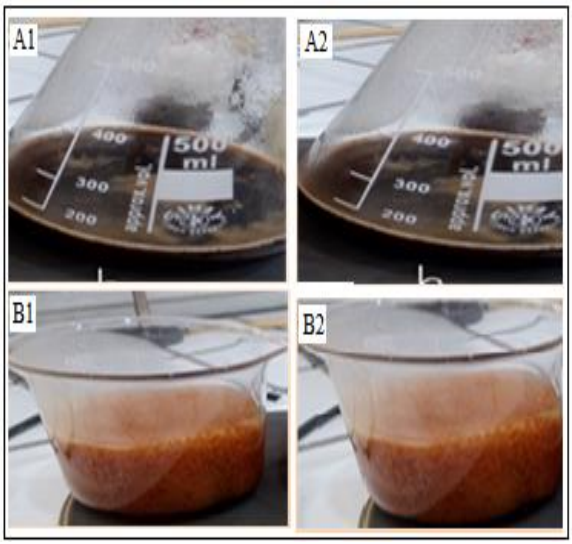


**Fig. 2.** Date syrups; Cultivars; A1: Hamraya65°C, A2: Hamraya105°C; B1: Litima65°C, B2: Litima105°C.

*Evaluation of antibacterial activity of date syrup*

The results noted in the present study show that the date syrups of the two cultivars Hamraya and litima illustrate a different antimicrobial effect depending on the concentration of the syrups and the pathology of the bacterial germs studied (sensitivity or resistance to some types of secondary metabolites) and to the type of bacteria (gram positive or gram negative). The results noted in the Table 3 and Table 4, show that date syrups prepared from cultivars Hamraya and Litime at different temperatures and high concentrations have an antimicrobial activity against gram-negative bacteria (*Pasteurellaspp, Pseudomonas aeruginosa)*, that gram-positive bacteria (*Staphylococcus aureus),* except Litime65.

However, *Escherichiacoli* is the most resistant strain for all syrups, this resistance is due to the composition of its cell wall, which is an outer membrane that consists of lipopolysaccharides that have an important role in the creation of a permeability barrier for most molecules (Saleh and Otaibi, 2013; Willey and *al*., 2013; Ghedadba and *al*., 2015).

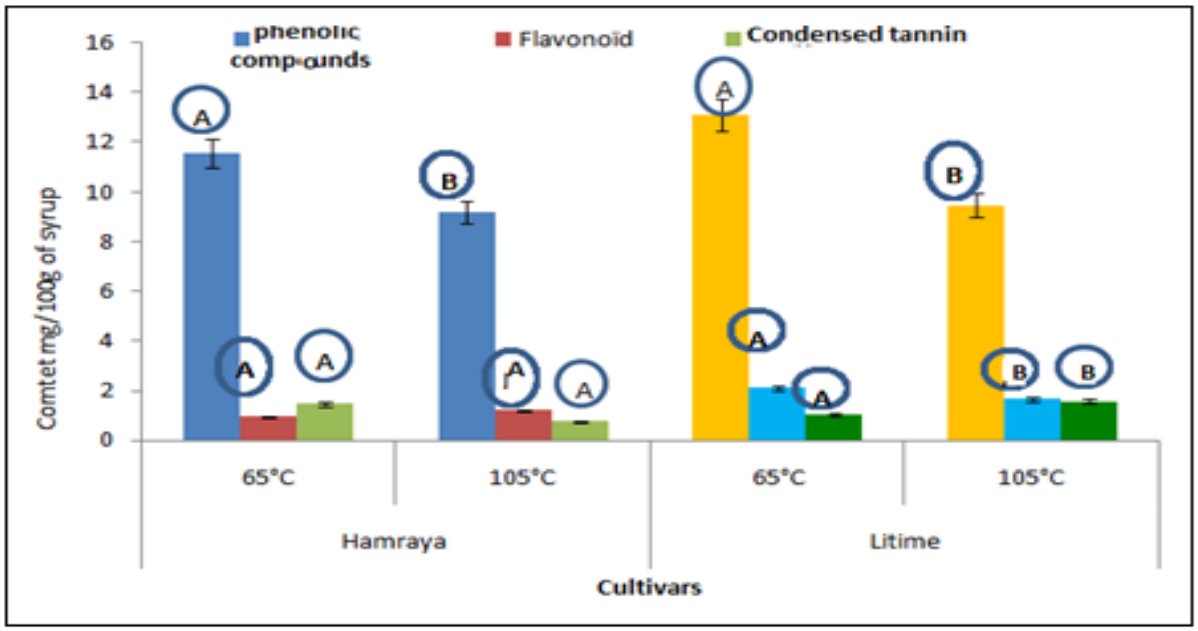


**Fig. 3.** Variation of the content of phenolic compounds, flavonoids and tannins in date syrups depending on the temperature.

These results are comparable to those found by Saci and Tliba, (2019). These authors showed the effectiveness of date extracts of Takermoust and Tamjouhert cultivars on gram-positive and gram-negative bacteria (*Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Streptococcus*Sp), they have inhibitory activity against bacterial growth. Antibacterial activity varies according to the strain. Gram-negative bacteria exhibit significant activity compared to Gram-positive bacteria. The results announced in this part of the present study mention the appearance of an inhibition zone of 9 mm for

gram-negative bacteria (*Pasturellaspp*), with Hamraya date syrup 65° C in 0.5 mg/ml concentration. Thus, Hamraya syrup 65°C shows a zone of 7 mm diameter for *Pseudomonasaeruginosa* gram-negative bacteria. At the same time, Hamraya cultivar syrup 105°C evokes a zone of 7 mm of 0.5 mg/ml of concentration for *Pasturella* (gram-negative bacteria).

An inhibition zone of 10mm of diameter is also observed at0.5mg/ml concentration of Litima105°C cultivar syrupagainst gram-positive bacterium *Staphylococcus aureus*, and an inhibition zone of 7mm for 0.5mg/ml concentration for *Pasturella spp*. The results found by Saci and Tliba (2019) shows also that *E.coli* is the most sensitive strain to date extracts with inhibition zones of 08 and 11.5 mm compared to gram-negative bacteria.Overall, of the above, the most sensitive bacterial germ is *Staphylococcusaureus* against Litima105 ° C and *Pasturellaspp* bacteria against Hamraya65 ° C syrup. These results can be explained by the difference in chemical composition between different syrups. The antibacterial activity of studied date syrups may be due to polyphenols and tannins that play an important role in protein precipitation and inhibition of microorganism enzymes (Nazand *al*., 2007). The involvement of phenolic compounds of Algerian date extracts in their antibacterial activity was confirmed in the work of Daasamiour and *al*. (2014).

In fact, the activity of date extracts on *Staphylococcus aureus* may be due to flavonoids that exhibit an antibacterial activity according to Bruneton (1999). Cushnie and Lamb (2011) report that flavonoids are inhibitors of sortases (enzymes found in cytoplasmic membrane of GRAM-positive bacteria) and that epigallocatechin impedes the secretion of coagulase and α-toxin.

**Conclusion**

The characterization of date syrups of the cultivars studied having low market value Hamraya and Litima, shows that the concentration at low temperature by evaporation is the best method of preparation. The adopted method is simple and less expensive, it can be applied even on household. The content of phytochemicals compound (soluble solids) transferred from dates to syrups seems not negligible. Date syrups of both cultivars show an interesting phytochemical composition and as well as an interesting antibacterial activity. Overall, these elaborate syrups can be integrated into the diet of the local population according to therapeutic properties conferred to them.

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