ETHNO-PHARMACOLOGICAL USE AND ANTIMICROBIAL ACTIVITY OF TRADITIONAL DATE VINEGAR OF GHARDAÏA

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Abstract.- *This study aims to demonstrate the therapeutic advantage of traditional date vinegar. We carried out an ethno-pharmacological survey on the therapeutic use of date vinegar by the population of Ghardaïa. After we verified the quality of two vinegars made from Deglet-Nour and Temjouhart date varieties, we tested their antimicrobial activity on* Staphylococcus aureus, Pseudomonas aeruginosa *and* Candida albicans. *These microorganisms are involved in skin infections treated with date vinegar. The ethno-pharmacological survey showed that the traditional date vinegar is used in the treatment of 30 afflictions. The treatment seems to be effective (75% of only positive effects compared to 14% of positive and also adverse effects). The antimicrobial activity was present, in spite of the weak concentration of acetic acid in the tested vinegars. The highest antimicrobial effect was observed on* S. aureus. *The inhibition diameter of this microorganism was 49 mm in Deglet-Nour vinegar and 33 mm in Temjouhart vinegar. In conclusion, date vinegar is used by the population of Ghardaïa to treat various illnesses and it was proven that it is effective in the treatment of skin infections caused by microorganisms mentioned previously.*

Key words: *date vinegar, ethno-pharmacology, antimicrobial activity, skin infections,* Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans.

USAGE ETHNO-PHARMACOLOGIQUE ET ACTIVITÉ ANTIMICROBIENNE DU VINAIGRE DE DATTES TRADITIONNEL DE GHARDAÏA

Résumé.- *Le but de cette étude est de démontrer l'intérêt thérapeutique du vinaigre traditionnel de dattes. Nous avons mené une étude ethno-pharmacologique sur le traitement par le vinaigre de deux variétés de dattes auprès de la population de Ghardaïa. En suite, nous avons vérifié la qualité de deux vinaigres fabriqués à partir des dattes de Deglet-Nour et de Temjouhart, puis testé leur activité antimicrobienne contre* Staphylococcus aureus, Pseudomonas aeruginosa *et* Candida albicans. *Ces germes sont impliqués dans les infections de la peau traitées par le vinaigre de dattes. Les résultats ont montré que le vinaigre traditionnel de dattes est utilisé dans le traitement de 30 affections. Le traitement semble être efficace (75% de résultats positif et sans aucun effet indésirable par rapport à 14% de résultats positifs mais avec des effets indésirables). L'activité antimicrobienne était présente, malgré la concentration faible en acide acétique dans les deux vinaigres. L'effet antimicrobien le plus fort a été observé contre* S. aureus *avec un diamètre d'inhibition de 49 mm chez le vinaigre de Deglet-Nour et de 33 mm chez le vinaigre de Temjouhart. On a conclu que le vinaigre de dattes est utilisé par la population de Ghardaïa pour traiter diverses maladies et il s'est avéré efficace dans le traitement des infections de la peau causées par les germes précités.*

Mots clés: *vinaigre de dattes, ethno-pharmacologie, activité antimicrobienne, infections de la peau,* Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans.

Introduction

The number of date palm (*Phoenix dactylifera* L.) in Maghreb is estimated at 177000 trees. Date palm provides a wide range of products with multiple uses. The most important are dates which the total production reaches 426.000 tonnes a year, then derivative products used in the traditional pharmacopeia. These products kept their importance in the oasis populations of Maghreb, in spite of the progress of medicine. This is due to their proven effectiveness for many years [1]. The dates have been used for the production of traditional vinegar in the oasis for a long time. It is an inherited practice with traditional material that provides a vinegar with therapeutic and flavor advantages which are absent in the industrial vinegar [2]. Indeed, the vinegar has been recognized for a long time to have astonishing therapeutic properties and recently, studies showed that the acetic acid, a vinegar component, is effective in a diagnosis of cervix cancer [3]. The objective of this study is to show the ethno-pharmacological uses of date vinegar, to investigate the vinegar quality and to prove its effectiveness in treating some infections by testing its *in vitro* antimicrobial activity.

1.- Materials and Methods

We collected, from Ghardaïa region, two vinegars produced from Deglet-Nour and Temjouhart dates. These vinegars were produced traditionally by washing and putting dates in a clean plastic barrel then filling two thirds of its volume with tap water. Old date vinegar and some salt were also added. Finally, the barrel was closed at least for forty days.

1.1.- The ethno-pharmacological survey

The ethno-pharmacological survey questionnaire was divided into three main questions about the knowledge of traditional medicine, date vinegar and what kind of medicine is preferred. The questionnaire was distributed on three categories of people: herbalists, traditional healers and the general population in order to obtain a maximum of information about the uses of date vinegar in traditional medicine in Ghardaïa oasis.

1.2.- Determination of vinegars quality

The pH of the vinegar samples was determined by using a pre-calibrated pH-meter. The rate of soluble solids, expressed in Brix degrees (°B), is the percentage of sucrose (w/w) in a pure solution [4]. It was determined by refractometer method. The dry matter was obtained by desiccation at 105 °C until a constant weight [4]. The concentration of the acetic acid was determined by titration with NaOH solution (0.5 N). The concentration of acetic acid is the concentration of NaOH multiplied by its volume and divided on the vinegar volume used in titration. The alcohol (v/v) remaining after making vinegar was deduced by comparing the acetic acid concentration found in vinegar to alcohol standard concentration (0.80 g in a volume of 1 ml). Thus, it is the acetic acid (w/v) multiplied by 1 ml and divided on 0.80 g. The alcohol is expressed in GL (ml of alcohol in a volume of 100 ml of vinegar) [5]. The total microflora was determined by colony counter method on nutrient medium incubated at 30°C for 48 hours [6].

All these analyses were duplicated and the results are presented as a mean.

1.3.- Test of the antimicrobial activity

The microorganisms tested were *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (IPA 200) which were conserved in ISP2 (International *Streptomyces* project 2) medium, and *Staphylococcus aureus* (ATCC 25923) which was maintained in conservation medium. The bacterium *Staphylococcus aureus* (ATCC 25923) was received from the university hospital of Blida. However, the bacterium *Pseudomonas aeruginosa* (ATCC 27853) and the yeast *Candida albicans* (IPA 200) were obtained from the collection of the Laboratoire de Biologie des Systèmes Microbiens (LBSM). All these microorganisms were stored at 4°C.

Microorganisms were plated in ISP2 medium (18 g agar /l) and incubated at 30°C [7]. After 24 hours, three colonies of each microorganism were homogenized in 9 ml sterile salt water. The antimicrobial activity of the non-diluted (pure) vinegars of Deglet-Nour and Temjouhart was realized. Furthermore, the antimicrobial effect of diluted two varieties of vinegars to 1/2, 1/4 and 1/8 with water, to determine the maximum dilution that should be employed in traditional mixtures, was also studied. In addition, the pure and diluted vinegars were tested in sterile (S) and non-sterile (NS) state with well and disc diffusion methods to highlight the probable antagonistic effect.

1.3.1.- Well diffusion method

A volume of 100 ml of ISP2 medium (12 g agar/l) was liquefied and inoculated with 80 µl of the microorganism suspension, then poured into Petri plates. After solidification of the medium, wells of 10 mm in diameter were punched and 100 µL of both sterile (S) and non-sterile (NS) vinegar dilutions was aseptically poured into the wells. The plates were kept for 4 hours at 4°C to allow the diffusion of the active substances while temporarily stopping the growth of the microorganisms. The plates were then incubated at 30°C. The diameters of the inhibition areas around the wells were measured after 24 hours of incubation for bacteria, and 48 hours for yeast [7].

1.3.2.- Paper disc diffusion method

Paper discs of 6 mm in diameter were sterilized by exposure to 254 nm ultra violet radiation for 45 min. After, they were soaked in the various dilutions of sterile (S) and non-sterile (NS) vinegars and deposited on the surface of ISP2 medium (12 g agar/l) which was previously inoculated with 80 µl of the microorganism suspension and poured into Petri plates. The plates were put at 4°C for 4 hours then incubated at 30°C. The diameters of the inhibition areas were measured after 24 hours of incubation for bacteria, and 48 hours for yeast [7].

The antimicrobial test experiments were duplicated and the results are presented as a mean.

2.- Results and Discussion

2.1.- The ethno-pharmacological use of date vinegar

The traditional date vinegar has 30 therapeutic virtues as shown in Table I, and it is used by 88% of the studied population sample, 7 herbalists and 2 traditional healers. The

treatment has shown positive effects on 75% of people who used date vinegar. However, it has shown positive and also adverse effects on 14% (fig. 1).

Table I.- The therapeutic virtues of date vinegar

Disease	Vinegar (date variety)	Mode of use
Sunstrokes	All varieties	Compress, massage, washing (water and/or vinegar), syrup (vinegar and honey)
Cough	Temjouhart, Takernennayt	Syrup (vinegar and honey or vinegar and liquefied sugar)
Fever	Temjouhart,Takernennayt	Massage, compress on the head and the belly
Migraine and headaches	Deglet-Nour, Temjouhart, Tafezwine, Ghars, Tazeggwaght	Compress, massage, drink, inhalation
Flu and cold	Deglet-Nour,	Syrup, fumigation
Skin diseases	All varieties	Massage
Vitiligo	All varieties	Massage
Fungal skin infections	Deglet-Nour	Massage
Dandruff and alopecia	Deglet-Nour, Takernennayt	Washing
Indigestion	Deglet-Nour	Vinaigrette, drink (vinegar and water)
Obesity	All varieties	Drink (vinegar and water)
Hypercholesterolemia	Deglet-Nour, Temjouhart	Drink (vinegar and water)
Respiratory allergies	Deglet-Nour	Drink (vinegar and water), vinaigrette
Feet fatigue	All varieties	Bath
Angina	Deglet-Nour	Syrup (vinegar and honey), gargling (vinegar and water)
Dizziness	Deglet-Nour	Drink (vinegar and water)
Flatulence	All varieties	Compress, drink (vinegar and water)
Rheumatoid arthritis	Deglet-Nour	Massage, compress
Tear gas damage	Deglet-Nour	Face wash
Hypertension	Deglet-Nour, Temjouhart	Massage
Eczema	Deglet-Nour, Temjouhart	Cataplasm (vinegar and henna)
Cutaneous abscesses, furuncle and wound inflammation	Deglet-Nour, Takernennayt, Tafezwine	Compress, bath
Tonic	Deglet-Nour, Temjouhart	Drink (vinegar and water)
Inflammation and infection of mouth and gum	Deglet-Nour, Temjouhart	Gargling (vinegar and water)

Burns (reduce pain and infection)	Deglet-Nour	Massage, washing
Fungal infection of toes	Deglet-Nour	Cataplasm (vinegar and henna)

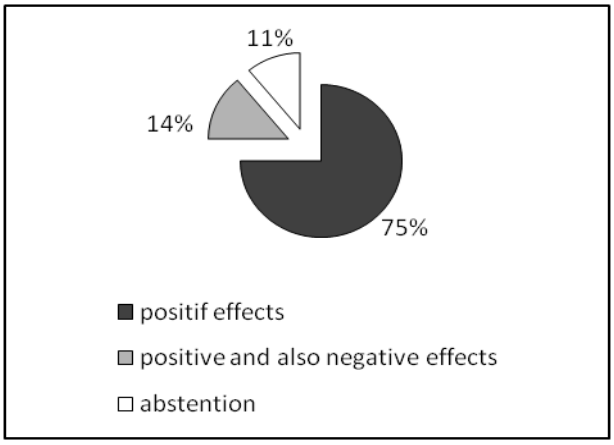

Figure 1.- The effectiveness of date vinegar.

Several therapeutic virtues shown by this study were also revealed by BALIGA *et al*. [8] on dates which are used in traditional medicine to treat hypertension, diabetes, cough, rheumatism, burning sensation, bronchitis, gastroenteritis, respiratory diseases, asthma, fever and fatigue. The fruit pulp is regarded to be antitussive, expectorant and tonic. Studies on rats showed that the defatted date seed flour reduced the plasma triglyceride, cholesterol and LDL. Other studies showed that the extract of date pulp and date seeds possess anti-inflammatory activity in the rat adjuvant arthritic model [8]. Moreover, the date vinegar has beneficial effects similar to those of other types of vinegars. Indeed, there is a close link between the risk to develop diabetes type 2 and excess weight. The vinegar has proven to be interesting in the prevention and the treatment of diabetes, because it decreases the presence of glucose and insulin in blood [9,10,11,12]. The acetic acid present in the vinegar is at the origin of the observed effects [11]. Moreover, the acetic acid increases the use of glucose by the human body [13]. Moreover, the vinegar increases satiety after the meal [9,10,11,12], and its daily consumption decreases blood pressure [14].

2.2.- Vinegars quality

The physico-chemical characteristics and the total microflora are presented in Table II and III. The results obtained in the analysis of our vinegars were compared with the studies of OULD EL HADJ *et al.* (2001) [2] and BOUAZIZ and OULD EL HADJ (2010) [15].

Table II.- The physico-chemical characteristics

	Deglet-Nour	**Temjouhart**
pH	3.44	3.28
Dry Matter (%)	13.31	12.26
soluble solids (°B)	13.75	9.65

Alcohol degree (°GL)	6. 29	2.22
Acetic acid (g/l)	50.3	17.8

Table III. The number of total microflora

Vinegars	**Total microflora (CFU/ml)**	
	Dilution 10^{-3}	**Dilution 10^{-2}**
Deglet-Nour	7×10^3	1.8×10^3
Temjouhart	6×10^3	1.1×10^3

The pH of Deglet-Nour and Temjouhart vinegars was slightly higher than the pH in H'Chef of Deglet-Nour (3.12) and in Harchaya (3.25) vinegars, but lower than the pH in Hamraya and Tinissine vinegars (3.65) [2,15]. Moreover, the dry matter rate obtained in our study was higher compared to 11.26% in Hamraya, 10% in H'Chef of Deglet-Nour, 6.59% in Harchaya and 6.33% in Tinissine [2,15]. In addition, the soluble solids rate was higher than 8.30 B in the H'Chef of Deglet-Nour, 10.00 B in Harchaya and 7.00 B in Hamraya [2]. However, our obtained values were lower than 16.53 °B obtained in H'Chef of Deglet-Nour [15]. The high soluble solids rate reveals a higher amount of unfermented sucrose. The Degree of alcohol in Deglet-Nour was much higher than 3.61 GL in H'Chef of Deglet-Nour, 3.75 GL in Harchaya, but lower than 4.90 GL registered in Hamraya [2]. However, the alcohol degree measured in Temjouhart vinegar was lower than other studied vinegars. Also, it was much higher than 0.99% (0.99°GL) in H'Chef of Deglet-Nour and Harchaya and 0.98% in Tachrwit and Tinissine [15]. The acetic acid concentration was higher in Deglet-Nour but lower in Temjouhart comparing to 25.94 g/l in H'Chef of Deglet-Nour and 30.38 g/l in Harchaya, but both vinegar concentrations were higher than 15.31 g/L noticed in Hamraya [2]. In addition, the acetic acid concentration of Deglet-Nour vinegar was higher than 0.9% (9 g/l) in Tinissine and 2.7% in H'Chef of Deglet-Nour [15]. In addition, the acetic acid concentration of Temjouhart was higher than 0.9% in Tinissine but lower than 2.7% in H'Chef of Deglet-Nour [15]. We should notice that among date vinegar quality characteristics found in this study, only the acetic acid concentration of Deglet-Nour follows the official Algerian standards for vinegar. Furthermore, the properties and the quality of traditional production of vinegar depend strictly on the conditions and varieties of dates used in the process.

2.3.- The antimicrobial activity

The obtained results showed that the bacteria *S. aureus* and *P. aeruginosa* were susceptible to Deglet-Nour and Temjouhart vinegars (figures 2, 3 and 4). However, the yeast *C. albicans* was susceptible only to Deglet-Nour vinegar (fig. 5). In addition, all the three studied microorganisms were more susceptible to Deglet-Nour by comparison to Temjouhart vinegar. Correspondingly, the studied microorganisms were more susceptible to non-diluted vinegar compared to diluted (1/2, 1/4 and 1/8) vinegar.

The bacterium *S. aureus* was susceptible to vinegar tested by both diffusion methods (wells and discs) as shown in figure 2 and 3. In addition, the similar results were obtained for *P. aeruginosa* and *C. albicans*. Only the results obtained by the disc diffusion method (for *P. aeroginosa*) and by well diffusion method (for *C. albicans*) were shown

(figure 4 and 5), respectively.

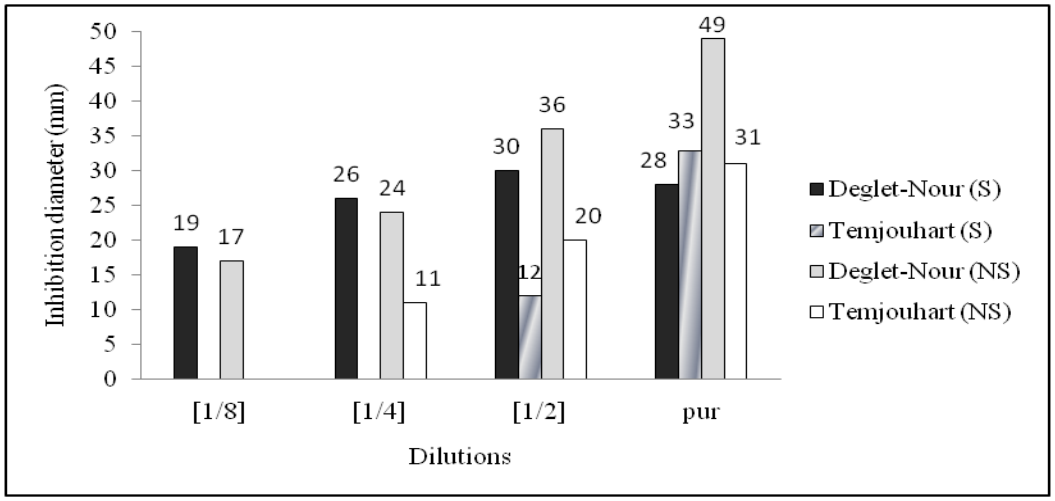

Figure 2.- Antibacterial effect on *Staphylococcus aureus* (well diffusion method) [S: sterile vinegar, NS: non-sterile vinegar]

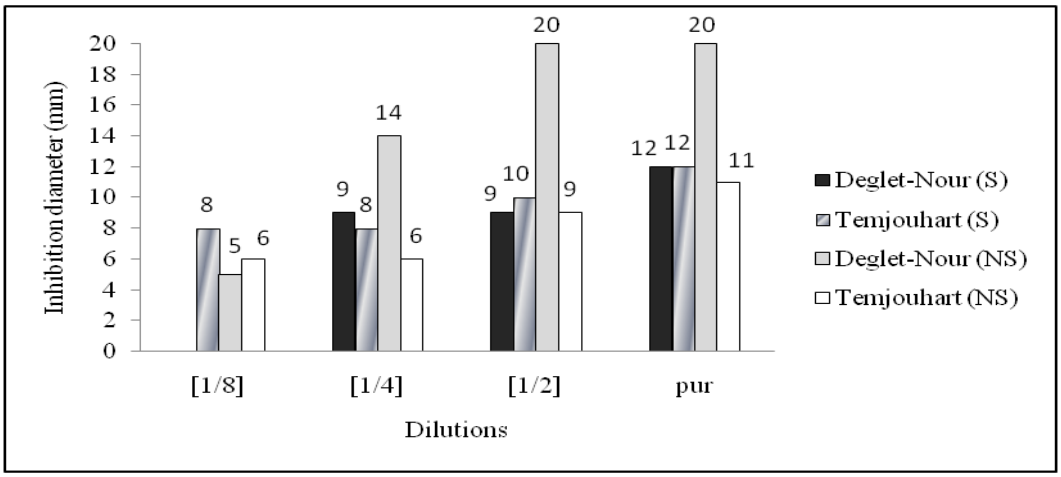

Figure 3.- Antibacterial effect on *Staphylococcus aureus* (disc diffusion method)

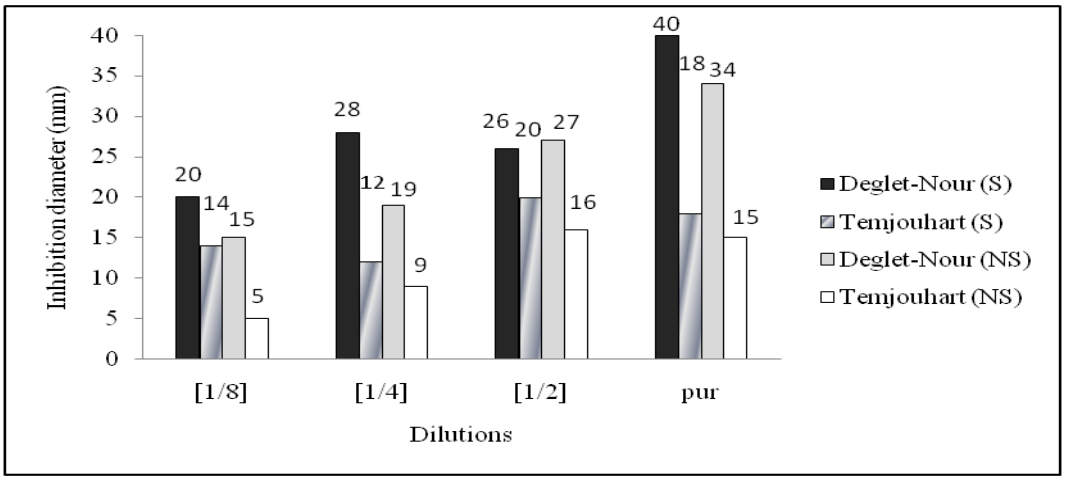

Figure 4.- Antibacterial effect on *P. aeroginosa* (disc diffusion method)

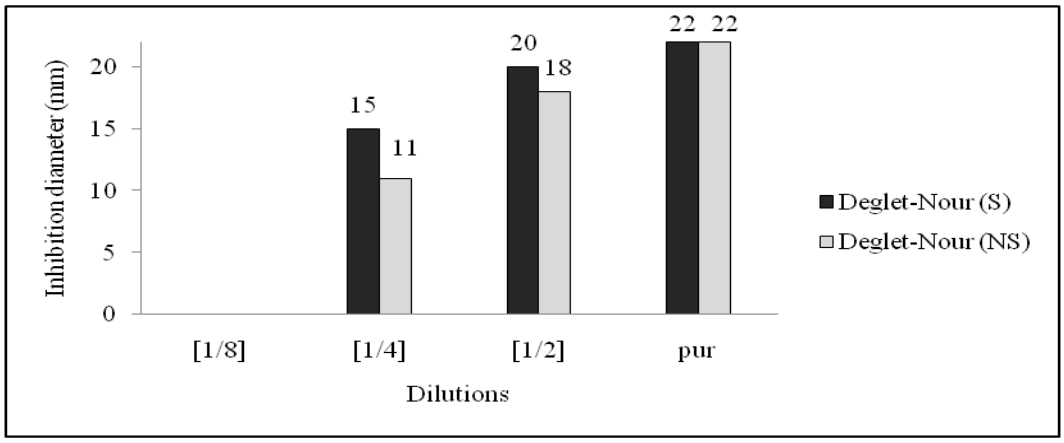

Figure 5.- Antifungal effect on *C. albicans* (well diffusion method)

In general, Deglet-Nour and Temjouhart vinegars exhibited more antibacterial than antifungal effect. Likewise, both varieties of vinegars showed less effect in weak concentration. Consistently, the commercial vinegar (35 g/l of acetic acid) revealed more antimicrobial effect on *S. aureus* and *Escherichia coli* than *Aspergillus niger* and *A. flavus*, and no antifungal effect was observed at low concentration [16]. Besides, vegetables washed by 30% and 20% vinegar solutions reduced significantly the microbial load to 1.10×10^7 CFU/ml, but a very low effect was observed by using vinegar solutions of 10% [17]. Similar results were noticed in our case by using 1/8 vinegar dilution. The result of our study suggests using 1/4 as the maximum dilution of date vinegar in traditional mixtures.

Furthermore, RUTALA *et al.* (2000) showed that white vinegar has antimicrobial activity against *P. aeruginosa*, both methicillin resistant and susceptible *S. aureus*, *S. choleraesius*, *E. coli* and against both vancomycin resistant and susceptible *Enterococcus* [18]. The vinegar has also an antibacterial activity against *Salmonella typhimurium* [19], and bamboo vinegar has an antimicrobial effect on fecal bacteria [20]. Moreover, THORP *et al.* (1998) showed that acetic acid was effective against the growth of bacteria that cause the most frequent earaches (Pseudomonas, Staphylococcus and Proteus) [21]. This proves that the acetic acid is responsible for the antimicrobial effect. We can explain this by the fact that organic acids diffuse passively through bacteria cell wall and dissociate into anions and protons. The release of protons decreases the internal pH which in turn provokes an inhibitory effect [22]. However, BALIGA *et al.* (2011) showed that *C. albicans* treated with high concentration of Barhi dates extract caused strong damage in the form of cell lysis, leakage of cytoplasmic material and eventual cell death and that flavonoids presents in the extract may have been responsible for the observed effect [8]. Besides, ALABBASY *et al.* (2013) showed that Zahdi date, Thompson seedless grape and maize malt vinegars contain flavonoids, vitamins and phenols [23]. One of the Zahdi date vinegar vitamins is the ascorbic acid [23] which KALLIO *et al.* (2012) found that it inhibited completely the growth of *S. aureus* and suggested that it affects the metabolism of *S. aureus* [24]. It increases the oxidative stress on bacterial cells when it is metabolized in aerobic conditions [24]. These results suggest that these phytochemicals could be present in Deglet-Nour and Temjouhart vinegars and were responsible for the observed antimicrobial effect.

S. aureus was more susceptible to Deglet-Nour non sterile vinegar. This may be due to the vinegar microflora antagonistic activity. RAJKOWSKA *et al*. (2012) observed that *Saccharomyces cerevisiae* var. *boulardii* has an antagonistic activity only against *S. aureus* rather than *Escherichia coli*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Campylobacter jejuni* [25]. However, VOHRA and SYAL (2013) showed that *Saccharomyces cerevisiae* has an antagonistic activity against *S. aureus, Pseudomonas* sp. and *Salmonella* sp. [26]. Moreover, *S. cerevisiae* has an antagonistic activity against *C. albicans, Protues, Shegilla* and *E. coli in vitro*, and antagonistic activity against *C. albicans*, *C. krusei* and *C. pseuditropicalis in vivo* which disappeared when the *S. cerevisae* was killed by heating [27]. Although *S. cerevisiae* is likely present in date vinegar, we recommend further studies to identify and isolate its microorganisms then test their antagonistic activity.

Conclusion

Date vinegar is largely used by the population of oases in Maghreb for its various therapeutic benefits, and each date variety provides vinegar with particular therapeutic effects. Date vinegar has a strong antimicrobial activity on *S. aureus*, *P. aeruginosa* and *C. albicans*. Therefore, it is effective in traditional medicine for treating skin infections caused by these microorganisms. Moreover, optimizing the traditional date vinegar quality will increase its effectiveness.

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