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Research Article

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Antioxidant activity of ethanolic extract of some pulp of fruit of *Zizyphus jujuba*, produces in the Algerian west, *in vivo* on *Saccharomycess cerevisea*

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ABSTRACT

Our aim consists to study the effect of phenoliques compounds some pulp of the fruit of zizyphus jujuba on the viability of saccharomycess cerevisea. To arrive to this end we beforehand isolated the stump of saccharomyces cerevisea on sabouraud middle. The effect of the ethanolic extract of zizyphus (EEZ) on the rate of survival of Saccharomyces cerevisiea showed that in the concentration of 176 mm of peroxide of hydrogen without EEZ nap of colonies is estimated in 221UFC / ml or a 50 % reduction corresponding to DL 50 while it attains the 344 (78 %) or a 28 % increase in comparison with the number of colonies in DL50. Our EEZ has a palliative effect by its antioxidant potential significant facing lethal effect of peroxide of hydrogen.

Key words: *saccharomycess cerevisea*, phenoliques compounds, *zizyphus jujuba* viability, antioxidant

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INTRODUCTION

Z. jujuba is kind most known by the fruits having the size of a nice olive. It is a polyvalent kind and its fruits, its leaves and its roots are of several interests on nutritious, cosmetic and curative plan.

Recent work has reported the antimicrobial Effects of Z.Jujube honey (honey Siddar) Tested on a range of germs that cause poisoning [1].

Also other works relate the value of this fruit in the treatment of cognitive decline [2], cancer [3][4] , inflammation [5]and in immunostimulation [6] [7].

Reactive kinds of the oxygen are well known as toxic agents capable of killing cells fast. Peroxide of hydrogen (H_2O_2), the anion superoxidizes (02-), and the radical hydroxyl ('OH) reactive kinds are which can harm a variety of cellular components drawing away the lipidic peroxydation, oxidation of proteins and of lesions of the DNA [8] [9]. The effects of these oxidising agents were involved in cancer, cardiovascular diseases and ageing; recently, they also showed an improvement of expression and replication of the HIV 1 [10] . The biological systems evolved several mechanisms of defence which allow the cells to face up lethal oxidising environments. These antioxidizing systems of defence include enzymatiques activities such as superoxidizes it dismutase and from the catalase who detoxifying oxidizers and protective molecules enzymatiques not including the glutathion, vitamins C and E and uric acid [11].
In this research we will determine the anti oxydent potential of the pulp of this fruit as potential source of bioactive natural molecules, allowing a protection against the effects of oxidising stress and development of the toxicity of synthetic antioxidizers.

EXPERIMENTAL SECTION

Materials vegetal:
Fresh Zizyphus, leaves were collected from the region of Sidi Bel Abbes in west Algerian in September, 2011. The plant material was identified according to African flowering plants database, and by local expert. A voucher specimen (#445) was deposited at the herbarium center of the department of Biology, Sidi Bel Abbes University (North West of Algeria) for future reference.

1. Preparation of antioxidants extracted from the jujube fruit (PFJ)
Phenolic compounds have been extracted from freshly prepared PFJ according to the modified method of Dewanto [12]. A sample of PFJ (50 g) was mixed with 150 ml of 80% ethanol for 05 min using a mixer kitchen. The mixture was homogenized in a vessel and extracted by sonication for 20 min. The supernatant was removed and the residue was subjected to a double re-extraction by repeating the above steps under the same conditions. The three filtrates were combined and filtered in Büchner then rinsed with pure ethanol. The alcoholic phase was evaporated under reduced pressure by a Rotary Evaporator Laborota 4000 at45°C. The ethanol extract of Ziziphus (EEZ) was stored at -20°C in the dark until used for the quantification of functional compounds.

2. Microbial samples collection
The isolation of yeast and preparation of circles Sabouraud For the bringing under cultivation of stocks and their isolation, the agar-agar of Sabouraud is used. This last is put in tubes in trial at the rate of 15 ml by tube and the pH is adjusted to 6.

3. Preparation of the range of peroxide of hydrogen
Calculation of the concentration of H_2O_2

The range is prepared from H_2O_2's solution of:
1-percentage massique 6 % (6 g H_2O_2 in 100 mL of solution)
2-molar Concentration: C = n/V
3-n = number of moles = mass / molar mass
A.N: 6 / 34.01 = 0,176 moles contained in 100 mL = 0,1 L of solution. That is 1,76 moles in

4. Resistance of *S. cerevisiea* in stress of peroxide of hydrogen in the presence of ethanolic extract of Ziziphus
To test the involvement of the raw extracts got in the answer to the stress caused by H_2O_2 we used the same insulated stump *Saccharomyces cerevisiea* before.

With determined DL50 before, we put our yeast on edge in the same conditions from a yest suspension in a middle YPD and in DO of 0.1 corresponding in a concentration of 1.3 10^6UFC / ML and a 100 % viability.

With this effect three preparations are performed as follows:

A tube witness containing 1,5 ml of a yeast suspension in some sterile physiological water (DO of 0.1) A second tube containing yeast suspension in 1.5 ml H_2O_2 (witness de la DL 50) A third tube containing 1ml raw extract of *Zizyphus jujuba* and 0.5ml of H_2O_2 in DL50.

After one hour the stress circles are remplaced with physiological water.

Different dilutions are performed allowing to get between 30 and 300 cells and from every tube 100µl every suspension are displayed on limp with Steeped in containing a complete middle YPG (glucose in 2 %, peptone in 1 %, extracts from yeast in 1 % and Agar in 1.5 %).
Colonies are counted at the end of 48 h.

RESULTS AND DISCUSSION

Study of the viability of *Saccharomyces cerevisiae*
1. Counting of colonies of *S. cerevisiae* after treatment with H_2O_2
After 48 h of incubation on complete middle YPD with 1,5 % Agar in 30°C, our colonies according to expression [N] were counted:

$[N] = (\Sigma c) / ((N1+0.1\ N2)\ dv)$

With:
Σc: number of all colonies calculated on limp them discreet
V: volume of inoculum applied in every limps; 100µL = 10-3ml
N1: number of limp discreet in the first dilution N1=3
N2: the number of limp discreet in the second dilution N2 = 3
d: rate of dilution of the first dilution kept for the counting on limps; d = 10-1

The calculation of percentage of survival from DO_{600}=0.1 corresponds in 1.3 10^6 UFC / ML and represents 100 % of survival. Got results appear in the figure 01.

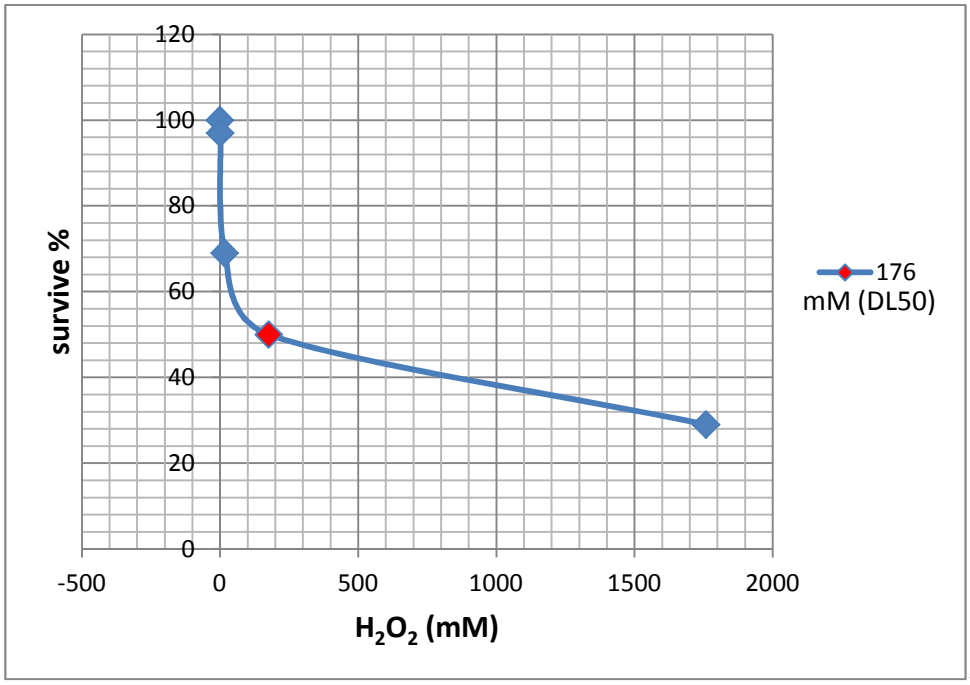

Figure 01: Count and calculations of colonies after treatment by peroxide of hydrogen

In of small concentration in the order of ≤17,6 mM toxicity is not important, and is without big influence on our stump.

In a 176 mM concentration count reveals a considerable reduction with less than 50 % of survival; it is DL50. It is the concentration kept for the second part of our job.

Beyond 176 mM mortality rate is very important and peroxide of hydrogen eliminates totality of *S. cerevisia*e.

2. Adaptation of *S. cerevisiae* to H_2O_2 in the presence of raw extract of PFJ

The second part of our job limits itself to test the viability of our stump in the concentration inducing to the mortality of 50 % of our yeast to know kept DL50 (176 mM).

After 48 h of incubation on complete middle YPD with 1,5 % Agar in 30°C. Colonies are counted according to the expression pointed out before [N].

The calculation of the percentage of survival from DO_{600}=0.1 which means 1,3 10^6 UFC / ml and representing 100 % of survival gives results regrouped in the figure 02.

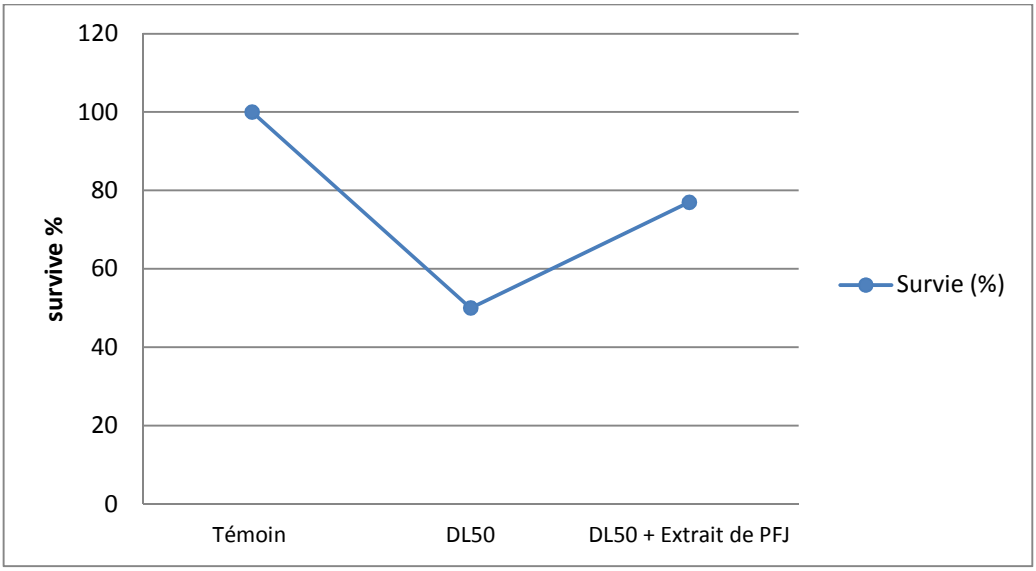

Figure 02: count of the colonies of *S. cerevisiae* and determination of their survive witness DL50 in the presence of raw extract of *zizyphus jujube*

The figure 02 illustrates the effect with the ethanolic extract of *Zizyphus jujuba* on the rate of survival of *Saccharomyces cerevisiea*. At 176 mM concentration without the extract of PFJ nap of colonies is estimated in 221 or a 50 % reduction corresponding to DL 50 while it attains the 344 (78 %) or a 28 % increase in comparison with the number of colonies in DL50.

These results show that our ethanolic extract of *Zizyphus jujuba* has a palliative effect facing lethal effect of peroxide of hydrogen. The antioxidant power of the extract of PFJ on oxydent effect of peroxide of hydrogen is important.

The yeast, being a cell in functioning and in metabolism comparable to that of human cells (such a true model representative of organisms eucaryotes), was always in the middle of basic research to include better cellular and genetic (human, animal and plant) phenomena. Besides, knowledge already acquired on the yeast (intrinsic qualities, resistance to difficult environments, nutritious needs) seems to give interesting perspectives in the domains of nutrition, of human or animal health and of energy [13].

CONCLUSION

Use of *S. cerevisiae* as model of study of the antioxidant potential of PFJ allowed further to a stress by of peroxide of hydrogen to determine DL50 of this yeast. Our ethanolic extract of *Zizyphus jujuba* has a palliative effect facing lethal effect in relation to *S. cerevisiae* of peroxide of hydrogen. The antioxydant power of the extract of PFJ on oxydent effect of peroxide of hydrogen is important. The different subsequent modifications in the presence of peroxide of hydrogen show up to some threshold means displayed by the yeast *S. cerevisiae* to struggle against oxydent effect. This struggle is of course made easier in our case by H_2O_2's neutralisation by the raw extract of the pulp of *Z. jujuba.*

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