

Analysis of phenolic compounds extracted from date of *Phoenix dactylifera* L (cultivar: Deglet Nour). search analgesic activity.

S. Ouafi and N. Bounaga

Research Laboratory arid, (LRZA) Faculty of biology,
USTHB. BPN ° 32 El-Alia, Bab Ezzouar, 16111, Algiers, Algeria.
Saida_ouafi@yahoo.fr

ABSTRACT

The date palm is a species particularly adapted to arid lands. It represents for the people of the Saharan oases indispensable species as it constitutes their resource base, especially for its fruit, date. In our work we were interested in extract, separation, purification and identification of polyphenols contained in the date of *Poenix dactylifera* L (cultivar: Deglet nour) of Adrar. The study of analgesic activity of these compounds in a second time. The extraction of large families of polyphenols, with different solvents (polar and non polar), allowed us to: -quantify by spectrophotometry UV-visible following compounds (flavones, flavonols: 1,12 mg / g \pm 0.14, anthocyanins: 1,75 mg / g \pm 0,43, C-glycosides: 2,286 mg / g \pm 0,29, and total phenols :3,6%) .Reveal and identify by thin layer chromatography (TLC), paper (CP), and high performance liquid chromatography (HPLC), the composition of each family. We have detected on HPLC profiles following compounds: as Flavone: Luteolin, Tricin, Chrysoeriol and 2 Flavonols: Quercetin, Isorhamnetin .The Cyanidin is the only anthocyanin isolated .The evaluation of analgesic activity occurs by injection of acetic acid intraperitoneally in mice that causes a painful reaction manifested by cramping, which can be reduced

by an analgesic product. This study compares the reduction in the number of cramps after administration of doses of test and reference product (Aspirin). Flavone C-glycosides and Anthocyanin extract of the date present a better analgesic activity than aspirin reference. The percentage of protection obtained from the results of "Cramping test" shows a significant activity of both extracts with the value (66.3%), which is double that obtained for the reference analgesic product (32.5%). The extract date is not only rich in phenolic compounds but they have a high analgesic activity.

Key words : date palm, date, flavonoids, sahara, cramps, analgesic

INTRODUCTION

The date palm or *Phoenix dactylifera* L. is the only species of the genus to be able to adapt to the hottest and driest regions in the world. At the base of the diet of saharan people the date palm is probably for agriculture in arids lands the best source (figure1).

In Algeria palm grove covers about 1 million hectares, almost all of the agricultural land areas located below the isohyet 100 mm / year (Sahki and Sahki 2004).

The number of palm is estimated at 17 million trees. It is the pillar of oasis agriculture and contributes for a large part to maintain the oasis biodiversity. A local and regional research in the field of flavonic chemistry has a certain

importance. It focused on the isolation and identification of the metabolic pathways of natural compounds that are of major importance in the biochemical mechanisms involved in the nutritional value of fruit: the date.

It is widely used by the local population as its therapeutic virtues. It is in this context that our work, is related, on the identification of the analgesic activity of flavonic content of fruit. This work is the first in Algeria.

MATERIALS AND METHODS

Plant material

The study was performed on 22 individuals of date from cultivar Deglet Nour harvested in the Biskra region (South-eastern Algeria) in 2012. The harvested plant material was kept cold and protected from light.

Animal material

The pharmacological test was performed on 40 albino mice (*Mus musculus*) having a weight of $20g \pm 5$.

Extraction of flavonoid aglycones

The method used was developed by Lebreton in 1967. It consists of hot acid hydrolysis (2N HCl) of cut plant material for 40 minutes in a water bath at 40°C. This hydrolysis allows the transformation of leucoanthocyanins to the corresponding anthocyanin and flavonoid aglycones released from their O-glycosides.

Extraction of glycosides

This technique was developed in 1973 by Harborne 1973. This is a maceration of plant material in a hydroalcoholic solution (70:30) to extract glycosids (O-glycosides and C-glycosides).

Spectrophotometric analysis

The quantitative evaluation of phenolic compounds (flavonols, flavones, anthocyanins and Glycosides) of 22 samples of dates is based on UV-visible spectrophotometric assay at 430 nm and 520nm.

Qualitative analysis by HPLC isocratic and gradient with solvents H²O – acetonitrile and methanol - acetic acid depending on the type of compounds (flavone aglycons, O- glycosides or anthocyanins).

Several chromatographic methods were used for the analysis of our extracts. The thin layer chromatography, high performance liquid chromatography, and UV-Visible spectrophotometry (Markham 1982).

Analgesic test

It consists of an evaluation of the analgesic activity of extracts of date palm dates by the technique of «Cramping test»(Vogel and Vogel, 1997).

After administration of the acetic acid to cause a painful reaction manifested by torsional movements of the abdomen with stretching of the hind legs (cramps).

The animals were fasted the night before the test. Thereafter, they receive through-gastric respectively 0.5mL of saline; reference product Feldene (20mg) and plant extracts (anthocyanins, glycosides and C-glycosides).

After 30 minutes, were injected intraperitoneally with 0.2mL of acetic acid 1% per mouse. After 5 minutes, counting cramps is achieved by direct observation for 10 minutes.

RESULTS AND DISCUSSIONS

The results obtained relate of the one part the biochemical study and biological activity of the other.

Results of the phytochemical analysis of date extracts

The extraction of large families of polyphenols, with different solvents (polar and non polar), allowed us to:

- Quantify by spectrophotometry UV-visible following compounds (flavones- flavonols: 1.12 mg / g \pm 0.04, anthocyanins: 1.75 mg / g \pm 0,065 C-glycosids: 1.08 mg / g \pm 0,029, and hétérosides 2.28mg/g \pm 0,176) .Total phenols 3.6%.
- Reveal and identify by thin layer chromatography (TLC), paper (CP), and high performance liquid chromatography (HPLC), the composition of each family. . We have detected on HPLC profiles following compounds: as Flavone: Luteolin, Tricin, Chrysoeriol and 2 Flavonols: Quercetin, Isorhamnetin .The Cyanidin is the only anthocyanin isolated . We found the same compounds that we isolated from date palm leaflets (Ouafi 2007, Ouafi and Bounaga 2010)

Results analgesic activity

The mean number of cramps are calculated for each batch. The results obtained are summarized in the table 1.

After counting cramps the percentage protection (figure 2) is calculated for each batch as follows:

Calculation of % protection :

$$\% \text{ of protection} = \frac{\text{Mean value of cramps of control beach} - \text{Mean value of cramps of E beach}}{\text{Mean value of cramps of control beach}}$$

According to the results, we note that the coefficient of protection of the reference product (Aspirin) is 32.5% meaning that the mice developed a more or less normal in reaction to the pain response.

By comparing the different results, we note that controls have a very low percentage of protection (0%) compared to the reference and testing. Which highlights the role of the drug used in reducing pain manifested by fewer cramps. The two test batches each have the same coverage rate of 66.3% for anthocyanins and heterosides.

1. Comparison between the percentages of protection : we set the null hypothesis H0: there is no significant difference between plant extracts and the reference product keywords.

We calculate two standard deviations: Since the percentage of equivalent protection for both extracts (butanol and ether) have a standard common type is calculated.

$\epsilon 9.6 > 1.96$: The H0 hypothesis is rejected, so there is indeed a significant difference between plant extracts and the reference product.

The use of Student's test, enabled us to conclude that the two plant extracts are significantly more effective than aspirin.

2. Discussion: the analgesic activity of polyphenolic extracts could be due to flavonoids. This can be explained by the ability of these to inhibit prostaglandins. These sensitize peripheral pain receptors to the action algogenic of other mediators (histamine and bradykinin). Blocking their synthesis will remove the effects of sensitization and reduces pain (Chauvelot-Moachon, 1988).

We can say that the phenolic compounds of the date of date palm tree have a strong analgesic activity which explains its use to relieve abdominal pain by the population of the southern regions of Algeria. (Cheriti et al. 2000).

CONCLUSION

Quantitative analysis by spectrophotometry UV-Visible of the date extract of date palm tree of Biskra, allowed us to evaluate

the mean values of various phenolic compounds (flavones, flavonols, anthocyanins, glycosides and C-glycosides flavone.

Finally, the analgesic activity of the date extract of cultivar Deglet Noor has been demonstrated so evident and statistically valid. It is due to anthocyanins and heterosides

Literature Cited

- Chauvelot-Moachon,, L., Brouilhet H., Giroud, J., 1988. Anti-inflammatoires Non Stéroïdiens, *in* Pharmacologie clinique, bases de la thérapeutique. *Expansion Scientifique Française*, 2^e ed., 709-742pp.
- Cheriti, A., 2000. Plantes médicinales de la région de Bechar, Sud-Ouest de l'Algérie : Etude ethnopharmacologique. the Algerian Sahara, *Flavour Fragr. J.*,22,286-288 pp.
- Harborne,J.B, 1973: phytochemical methods, guide to modern technique of plant analysis Chapman and Hall, London 378.
- Lebreton, PH., Jay M., Voirin,B. et Bouchez M.P.,1967. Sur l'analyse qualitative et quantitative <<<<des flavonoïdes. Ed. n°7 *Chimie analytique*, 49, p375-383.
- Markham, K.R., 1982. The techniques of flavonoids identification. *Academic press*, London, 313p. *In* Andersen M.Ø., Markham K.R., 2006. Flavonoids: Chemistry, biochemistry and applications. *CRC Press*, USA, 1197 p.
- Ouafi, S., 2007. Contribution à l'étude biochimique du Palmier dattier, analyse des polyphénols des folioles de palme de cultivars du *Phoenix dactylifera* L : Dans un but chimiotaxonomique. Thèse d'état : science de la nature, physiologie végétale, USTHB, Alger, 140p.
- Ouafi, S.,and Bounaga,N., 2010 .Phenolic compound as marker for Algerian cultivars of the date palm *Phoenix dactylifera* L. ISHS Acta Horticulturae 882: IV International Date Palm Conference.
- Sahki, A. and Sahki, R, 2004 : le Hoggar promenade Botanique, édition Esope p247.
- Vogel, H.G. and Vogel,W.H., 1997. Drug Discovery and Evaluation. *Pharmacological Assays*, 382 p.

Table

Table 1. Results count cramps for each batch

Number cramps / Mouse	Controls	Reference (Aspirin)	Anthocyanins -C-glycosids (Test 1)	Heterosids (Test 2)
Mean value	21± 3,90	10,8± 0,6	5,4 ± 0,3	5,5± 0,3
% of protection	0 %	32,5%	66,3%	66,3%

Figures

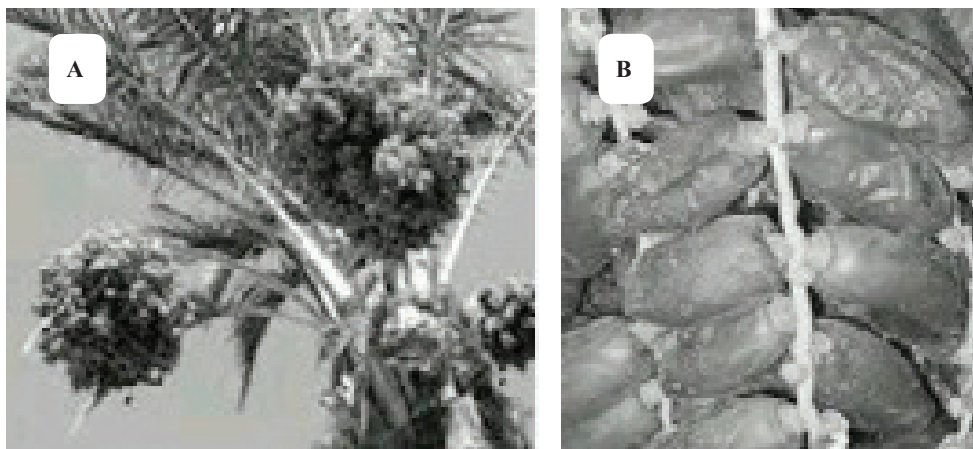


Fig .1. Date palm tree (A) and dates of cultivar Deglet Nour(B).

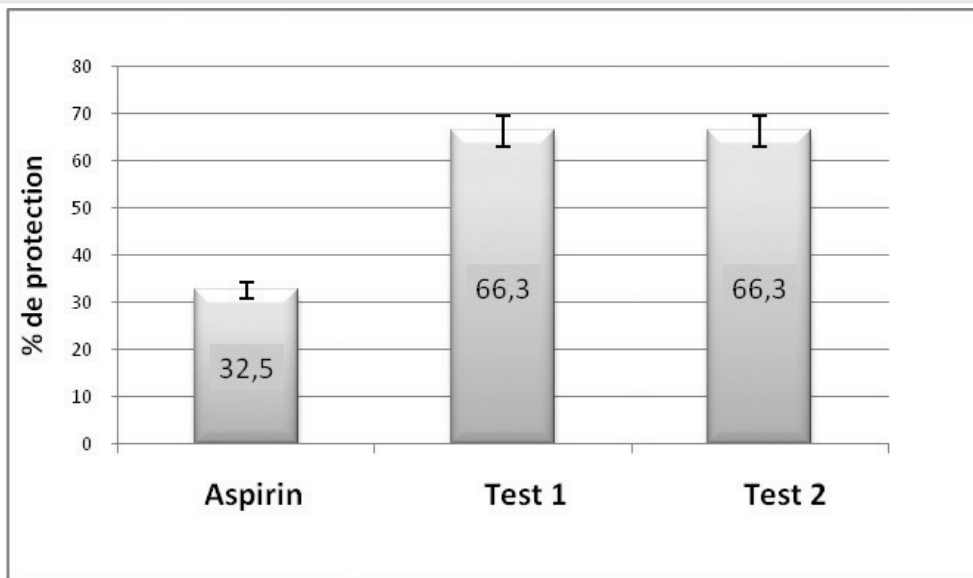


Fig. 2. Histogram showing the percentages of protection of the three test batches