CHEMICAL COMPOSITION AND ANTIOXYDANT PROPERTIES OF *WASHINGTONIA FILIFERA* LEAVES AND FLOWERS

Saoussen HAMMAMI[a], Saoussen Ben Salem[b], Hella Jarraya[b], Prabha DEVI[c], Adel NEFZI[d], Zine MIGHRI[a*]

[a] *:* Unité de Recherche12-04, Chimie Appliquée et environnement, Faculté des Sciences de Monastir

[b] *: Laboratoire de Chimie des Substances Naturelles et de Synthèse Organique (99/UR/12-26), Faculté des Sciences de Monastir, 5019, Monastir, TUNISIE*

[c]*: Bio-Organic Chem Lab, National Institute of Oceanography, Dauna Paula, GOA, 403004.*

[d]*: Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port St. Lucie, FL 34987, USA*

(Reçu le 24 Janvier 2011, accepté le 12 Mai 2011)

RESUME: Le présent travail décrit une étude de la composition chimique et des effets antioxydants d'extraits bruts et volatils des feuilles et des fleurs de l'espèce *Washingtonia filifera (W.H)*. L'évaluation des pouvoirs antioxydants a été effectuée en se basant sur la méthode impliquant le radical 2,2-diphényl-1-picrylhydrazyle (DPPH). Cette évaluation nous a permis de constater que les extraits butanolique et éthanolique des feuilles et des fleurs du palmier possèdent des pouvoirs antioxydants moyennement significatifs et qu'ils peuvent être considéré comme source naturelle d'antioxydants potentiellement protecteurs. Des études chimique et chromatographique des extraits actifs, nous a permis d'isoler et d'identifier le dérivé triacétylé du Tricine **1** et le composé hétérocyclique et biologiquement actif Quinazoline-*2,4-(1H, 3H)* dione **2** signalés pour la première fois dans l'espèce *Washingtonia filifera (W.H)*. Leurs structures ont été élucidées par le moyen de la RMN (^{1}H et ^{13}C) 1 et 2D consolidée par la spectrométrie de masse en mode ES.

Mots clés : *Washingtonia filifera (W.H*), activité antioxydante, Tricine triacétylé, Quinazoline-*2, 4-(1H, 3H)* dione.

ABSTRACT: The chemical composition and antioxidant effects of extracts from *Washingtonia filifera* leaves and flowers were studied. Antioxidant testing assay has been done on the basis of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Among all tested extracts and the volatile fractions, butanol and ethanol ones from *Washingtonia filifera* leaves and flowers, were the most potent ones, the observed results indicate that they may be considered as a natural source of antioxidants having health protective potential. Chemical investigation of these extracts, led to the isolation and the identification of Tricine acetylated derivative **1a** and of synthetic heterocyclic bioactive Quinazoline-2, 4-*(1H, 3H)* dione **2** mentioned for the first time in *Washingtonia filifera (W.H)*. Structures were assigned on the basis of spectroscopic data, especially (^{1}H and ^{13}C) 1 and 2D NMR and ES Mass spectrometry.

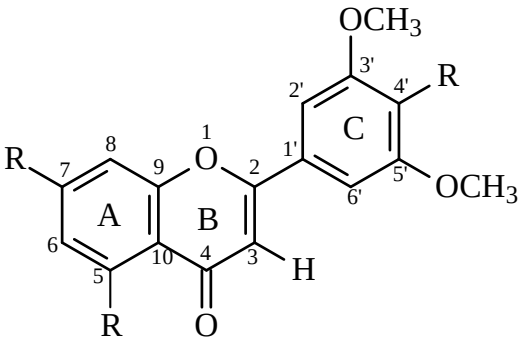
Key words: *Washingtonia filifera (W.H*), antioxidant effects, Tricine triacetates, Quinazoline-*2, 4-(1H, 3H)* dione.

INTRODUCTION

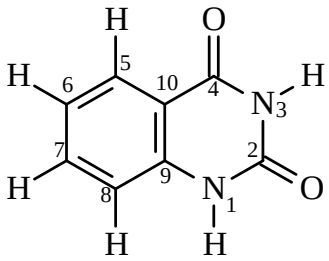
Surrounded by natural beauty, actually, most of the scientists are trying to valorise more and more the flora and fauna as natural resources of bioactive interesting products by studying their offensive and defensive effects. The Palm family is one of the most useful families of plants widely used as food, as fertilizers and in folk medicine. *Washingtonia filifera (W.H)* is one of the palm tree native of south California, Arizona, Mexico and desert zones *[1]*, it is resistant to the cold. Phytochemicals previously isolated from *Washingtonia filifera (W.H)* specie are flavonoid glycosides, gibberlic and abscissic acids *[2-3]*. As a part of our ongoing research on biologically active compounds of pharmaceutical and economical importance *[4-8]*. Chemical investigation of

* corresponding author, e-mail: zinemighri@yahoo.fr

polar extracts deriving from *Washingtonia filifera (W.H)* led to the isolation and the identification of tricine derivative and the heterocyclic Quinazoline-*2,4-(1H, 3H)* dione isolated for the first time from this specie. We remind that quinazoline-*2,4*-dione derivatives are generally known as heterocyclic compounds having versatile and antihypertensive activities as well as anticonvulsant activity against electroshock. Moreover, they show useful anti-inflammatory property and cause vasodilatation in animals *[9-10]*.



Compound 1, R=OH
Compound 1a, R=OAc



Compound 2

RESULTS AND DISCUSSION

Compound 1, structure of compound **1** has been identified through its acetylated derivative **1a**. An ESI-HRMS of **1a** gave a molecular ion at m/z 456.9199 $[M+H]^+$ corresponding to a molecular formula of $C_{23}H_{20}O_{10}$. Structure elucidation was deduced through the extensive use of nuclear magnetic spectroscopy experiments including COSY, CHCorr and ^1H-^{13}C experiments (Table I). The ^1H-NMR spectrum displayed typical signals of Tricine nucleus *[11]*. In fact one pair of doublets with a relatively weak coupling constant (J_{meta} 2.4 *Hz*) observed at δ_H 6.85ppm and δ_H 7.33 ppm indicates the presence of a tetra substituted aromatic ring (nucleus A). The second symmetric and tetra substituted aromatic ring C, has been deduced by the observation of one singlet (2H) appearing at δ 7.07ppm. In addition, characteristic signals from three phenol acetoxy groups (2.40ppm-2.50ppm) suggested that **1a** is a triacetates derivative. The presence of two methoxyl groups (δ 3.90 ppm) directly linked to $C_{3'}$ and $C_{5'}$ in Tricine C ring, was evidenced by H_3-C_2, H_3-C_4, H_3-$C_{1'}$, $H_{2'}$-C_2, H_{11}-$C_{3'}$ and H_{12}-$C_{5'}$ long range couplings deduced from HMBC spectrum. Therefore, compound **1a** was identified as Tricine triacetate derivative. The corresponding natural compound is reported for the first time in *Washingtonia filifera (W.H)* specie.

Table I: ^{13}C (75 *MHz*, $CDCl_3$) and ^1H (300 *MHz*, $CDCl_3$) spectral data of compound 1a

Atom	δ ^{13}C	δ ^{1}H	*multi.*	*J* (*Hz*)
1	-	-	-	-
2		-	-	-
3	103.6	6.60	s	-
4	176.6	-	-	-
5		-	-	-
6		6.85	d	2.4
7		-	-	-
8		7.33	d	2.4

9		-	-	-
10		-	-	-
11; 12				
1’		-	-	-
2’; 6’	114.2	7.07	s	-
3’; 5’	156.3	-	-	-
4’		-	-	-

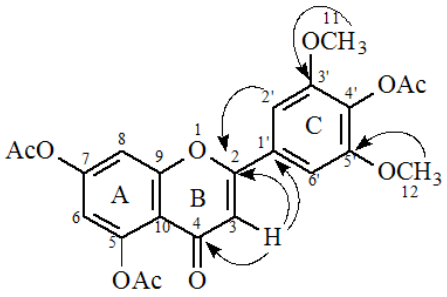


Fig.1 Significant ^{1}H-^{13}C long range couplings for **1a**

Compound 2

Positive HR-ES mass spectrum of compound **2** showed a pseudo-molecular ion $[M+H]^+$ at m/z 162.9109 compatible with the molecular formula of $C_8H_6O_2N_2$. Its ^{1}H NMR spectrum recorded at 300 *MHz* in $CDCl_3$ displayed typical signals of an ortho disubstituted aromatic ring. ^{13}C and DEPT 135 NMR spectra showed eight signals, four of which are attributable to aromatic methane groups; the others are relative to quaternary carbons (δ 155.6ppm-166.7ppm). Characteristic chemical shifts of the two carbons C_4 (δ 166.8ppm) and C_2 (δ 116.4ppm) indicated the presence of two carbonyl groups in the structure. Comparison of ^{1}H and ^{13}C NMR data of compound **2** (Table II) with those reported for quinazoline-*2,4*-dione derivatives *[9,10,12]* suggested that **2** is Quinazoline-*2,4-(1H, 3H)* dione, reported for the first time in *Washingtonia filifera (W.H)* specie. This was approved by the significant longue range correlations H_8-C_{10}, H_8-C_6, H_5-C_9, H_7-C_9 and H_5-C_4 observed in ^{1}H-^{13}C HMBC spectrum.

Table II: ^{13}C (75 *MHz*, $CDCl_3$) and ^{1}H (300 *MHz*, $CDCl_3$) spectral data of compound 2

Atom	δ ^{13}C	δ ^{1}H	*multi.*	*J (Hz)*
1	-	-	-	-
2	166.6	-	-	
3	-	-	-	-
4	166.2	-	-	
5	125.5	7.81	dd	8.4 ; 2.1
6	125.7	7.24	m	-
7	134.5	7.52	td	7.8 ; 1.8
8	117.9	7.24	m	-
9	155.6	-	-	-

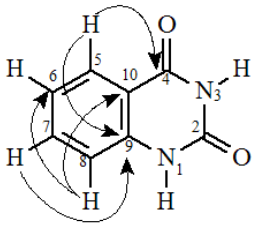


Fig.2 Significant ^{1}H-^{13}C long range couplings for **compound 2**

Antioxidant activity. Tables **III** and **IV** lists the antioxidant activity of crude extracts from leaves and flowers compared to quercetin used as standard compound. EC_{50} permitted the evaluation of antioxidative effects. The potential antioxidant activity is higher for extracts having lower EC_{50} values. We noticed according to results represented in table III, that ethanol and butanol polar extracts from flowers and leaves of ***Washingtonia filifera*** exhibited the strongest antioxydative power (EC_{50}=0.52, for ethanol extract and 0.99 for butanol one). Figures **I** and **II** presented the variation of inhibition percentages of DPPH within concentrations of extracts from leaves and flowers of ***Washingtonia filifera*** palm tree. We noticed that reducing power increased while

increasing concentration. The most polar extracts had the highest ability to reduce DPPH and their antioxidant effects could be related to the presence of phenolic compounds and flavonoids *[13, 14]*

Table III: Free radical scavenging capacity in DPPH system (IC_{50}, $mg.mL^{-1}$) of different extracts from *W. Filifera* leaves

***Washingtonia filifera* flowers**	
Petrolium ether	1.5
Chloroform	1.1
Butanol	**0.99**
Volatile extract	1.4
Standard	
Quercetin	$4.6x10^{-3}$

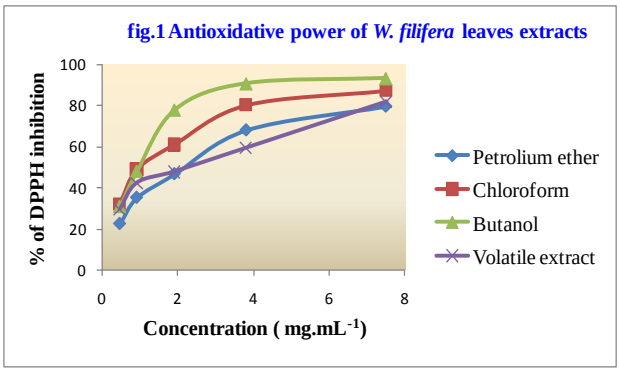
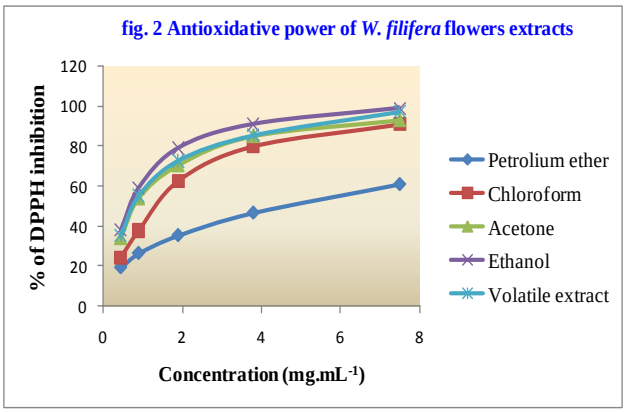


Table IV: Free radical scavenging capacity in DPPH system (IC_{50}, $mg.mL^{-1}$) of different extracts from *W. Filifera* flowers

***Washingtonia filifera* flowers**	
Extract	EC_{50} ($mg.mL^{-1}$)
Petrolium ether	3.91
Chloroform	1.1
Acetone	0.71
Ethanol	**0.52**
Volatile extract	0.61



EXPERIMENTAL

All NMR experiments were performed in $CDCl_3$ and CD_3OD on a Bruker AC-300 NMR spectrometer using the solvent as internal standard. Silica gel (70-200µm) for CC and silica GF254 were products of sds, FRANCE.

3.1. *Palm material* Flowers and leaves of *Washingtonia filifera (W.H)* palm tree were collected on 26 of July 2008, from Monastir region (TUNISIA). A voucher specimen (W185) is deposited at Natural Substances and Organic Synthesis Laboratory at the Faculty of Science of Monastir.

3.2. *Extraction and isolation*

Preparation of crude extracts from *Washingtonia filifera (W.H)* leaves:

Fresh leaves of *Washingtonia filifera (W.H)* (680g) were submitted to an hydrodistillation giving an odorant distillat and an aquous residue which was successively extracted with petrolium ether, chloroform and butanol to yield three crude extracts.

Isolation of compound 1a

The polar leaves butanolic extract (1g) thus obtained, has been acetylated at room temprature with acetic anhydride in pyridine and then chromatographed on a silica gel column eluted with CH_2Cl_2/Acetone (9:1 to 0:10) gradient, to obtain 212 fractions of 15mL, regrouped on eight main groups (W_1-W_8) after TLC analysis, the sixth group (267mg) was choosen for a repeated separation by CC using the same gradient of solvent yielding five sub-fractions (Y_1-Y_5) having different chemical composition. Purification of the major constituent of the forth one (Y_4), gave 10mg of **compound 1a** as a white solid.

Preparation of crude extracts from flowers:

A sample of 720g of air-dried flowers was extracted at room temprature successively with petrolium ether, chloroform, acetone and ethanol. Concentration of the organic solutions thus obtained to dryness under vacuum, afforded four crude extracts.

Isolation of compound 2

Ethanolic crude extract (1.2g) from *Washingtonia filifera (W.H)* flowers, was subjected to CC (silica gel, 70g) eluting with methane dichloride gradually increased with acetone. According to TLC monitoring, 11 groups of fractions were collected (K_1-K_{11}). Fraction K_8 (309mg) containing the major interesting constituent was repeatedly chromatographed over silica gel column utilizing methane dichloride gradually increased with acetone, yielding 5 sub-fractions (F_1-F_5). The second one (79mg) was further concentrated under vacuum and purified by recristallisation in petrolium ether to afford 16mg of **compound 2** as a white solid.

Diphenyl-1-picryl-hydrazyl (DPPH) by Blois method

The DPPH radical-scavenging activity was assayed on the basis of Blois method *[15]*. A spectrophotometric method was used for the measurement of reducing power, after 30min of incubation, the absorbance of each solution was measured at 517nm. At least five different concentrations were prepared for each tested sample. $DPPH^{\bullet}$ Solution (4mg/100mL ethanol) served as the control.

The ability of the extract to scavenge DPPH was calculated using the following equation:

DPPH radical-scavenging activity % = [(Absorbance of the control-Absorbance of the sample)/Absorbance of the control]x100

EC_{50} = Concentration of antioxidant (test sample) required for a 50% decrease in absorbance of $DPPH^{\bullet}$

REFERENCES

[1] H. L. Oppenheimer and R.T. Bartletti, *Bishop Mus. Occas. Pap.*, **2002**, *69(2)*, 1-14.
[2] M. I. Khan, *Pakistan Journal of Botany*, **1998**, *30(2)*, 245-252.
[3] N. H. El-Sayed, N.M. Ammar, S.Y. Al-Okbi, A.L.T. El-Kassem, T.J. Mabry, *Natural Product Research*, **2006**, *20(1)*, 57-61.
[4] H. Ben Jannet, A. Chaari, A. Bakhrouf and Z. Mighri, *Natural product Research*, **2006**, *20(3)*, 299-304.
[5] H. Ben Jannet, L. Sakka Rouis, M. Neffati and Z. Mighri, *Journal of essential oil and bearing plants*, **2007**, *10(2)*, 151-156.
[6] A. Bergaoui, S. Hammami, M. Ben Halima Kamel, L. Sakka Rouis, O. Boussada, D. Houas and Z. Mighri, *Journal of Enthomology*, **2008**, *5(4)*, 277-283.
[7] S. Hammami, H. ben Jannet, A. Bergaoui, M.L. Ciavatta, G. Cimino And Z. Mighri, *Molecules*, **2004**, *9*, 603-607.
[8] S. Hammami, H. Ben Jannet, M.L. Ciavatta, G. Cimino and Z. Mighri, *Natural product Research*, **2007**, *21(8)*, 692.
[9] G. Liu, *Acta Cristallographica*, **2008**, 1677-1683.
[10] H. Yanling, L. Cuifen, C. Zuxing and Y. Guinchun, *J. Heterocyclic Chem.*, **2007**, *44*, 1421-1424.
[11] B. Garcia, J.A. Marco, E. Seoane and A. Tortajad, *Journal of Natural Products*, **1981**, *44 (1)*, 111-113.
[12] R. L. Sharma, J. Singh, S. Kumar, K. Daljeet, S. Anand, S. Poonam and J. Bhawana *Heterocyclic Chem.*, **2007**, *44*, 1501-1504.
[13] E. Yesilada, K.Tsuchiya, Y. Takaishi and K. Kawazoe. *J. Ethnopharmacol*, **2000**, *73*, 471-478.
[14] M. Ramezani, H. Hosseinzadeh and N. Daneshmand, *Fitoterapia*, **2001**, *72*, 255-262.
[15] M. S. Blois, *Nature*, **1958**, *181*, 1199-1200.