Journal of Fundamental and Applied Sciences

ISSN 1112-9867

Available online at http://www.jfas.info

Research Article

EXTRACTION OF ANTIOXIDANT PHENOLIC COMPOUNDS FROM ALGERIAN INULA VISCOSA LEAVES: INFLUENCE OF THE HARVEST PERIOD AND OPTIMIZATION

S. Amrouche*, F. Mohellebi, R. Derriche

Ecole Nationale Polytechnique, Laboratoire de valorisation des Energies Fossiles, 10 Avenue Hassen BADI BP182 El-Harrach 16200 Alger Algérie

Received: 03 December 2018 / Accepted: 20 August 2019 / Published online: 01 September 2019

ABSTRACT

The aqueous extraction of polyphenols from Inula viscoa leaves was carried out on two different lots in order to study the influence of the picking period on the phenolic content and on the antioxidant characteristics of the extracts obtained, as well as on the optimization of technological parameters. For the two lots studied, the simple effect of temperature, the solid to liquid ratio and the interaction effect between temperature and solid to liquid ratio are the most influential parameters on all recorded responses. The phenological cycle of the plant affects the amount of phenolic compounds recovered, as well as the antiradical power and the antioxidant characteristics. The multi-response optimization of the extraction conditions gives polyphenol contents of 291 and 251 mg GAE. g^{-1} DW and antiradical activities of 90 and 89% for the purified extracts of July and December, respectively.

Keywords: *Dittrichia viscosa* (L.) ; aqueous extraction; polyphenols ; Antiradical activity ; optimization

Author Correspondence, e-mail: sara.amrouche@g.enp.edu.dz

doi: http://dx.doi.org/10.4314/jfas.v11i3.11

1. INTRODUCTION

In recent years, aromatic and medicinal plants have attracted a great deal of interest in various fields, particularly in therapeutic medicine, since they contain a mixture of different chemical compounds that can act individually or in synergy to cure diseases and improve health. [1]. In fact, a single plant can have a variety of phytochemicals. Phenolic compounds are part and are becoming increasingly important and are attracting the attention of researchers and industries to substitute synthetic antioxidants that may have carcinogenic effects.

These molecules synthesized by plants and belonging to their secondary metabolism are grouped into a class composed of about 8000 compounds [2] Flavonoids are the largest group of phenolic compounds, accounting for more than half of the natural phenolic compounds (4000 flavonoids are found in the plant kingdom) [3]

Due to its geographical location and the diversity of its climate, Algeria is a country rich in natural substances including the inula viscosa [synonym: Cupularia viscosa or Dittrichia viscosa (L)] Greuter [4]. This perennial belonging to asteraceae family , frequent in the Mediterranean region has a very diverse therapeutic history and used since ancient times in traditional medicine, its therapeutic and antiseptic activities are numerous but they remain exploited in a traditional way. As for the phenolic compounds identified in the inula viscosa, recent research reveals the predominant presence of flavonoids [5], sesquiterpene acids [6,7,8] and triterpene esters [9,10]

Today, political, economic, societal and environmental issues also encourage manufacturers to innovate technologically and improve the energy and environmental efficiency of their processes, thanks to the development of so-called green chemistry. In this context, we opt for a solid-liquid extraction of phenolic compounds from inula viscosa using water as extraction solvent: a green solvent, safe, inexpensive and accessible.

However, compared to other plants, few studies and scientific research have focused on the inula viscosa. Moreover, plant matter is an evolutive matter whose solute content can only be guaranteed at a given moment for a given batch. Based on these considerations and in order to exploit the plant potential in Algeria, the present study was undertaken with the main objective of studying the influence of the collection period on the phenolic content, on the antioxidant characteristics of recovered extracts and on the optimization of technological parameters and the formal modeling of the extraction phenomenon.

2. EXPERIMENTAL

2.1 Plant material

The lots of the inula viscosa used were collected in July and December 2014 in Ben Aknoun located 6 km southwest of the city of Algiers (Algeria), at 270 meters above sea level. The used leaves were dried in a dark ventilated room to increase life span and inhibit enzymatic degradation and bacterial growth. The leaves are crushed to an average particle size of 237 μm and have a moisture content of 2%.

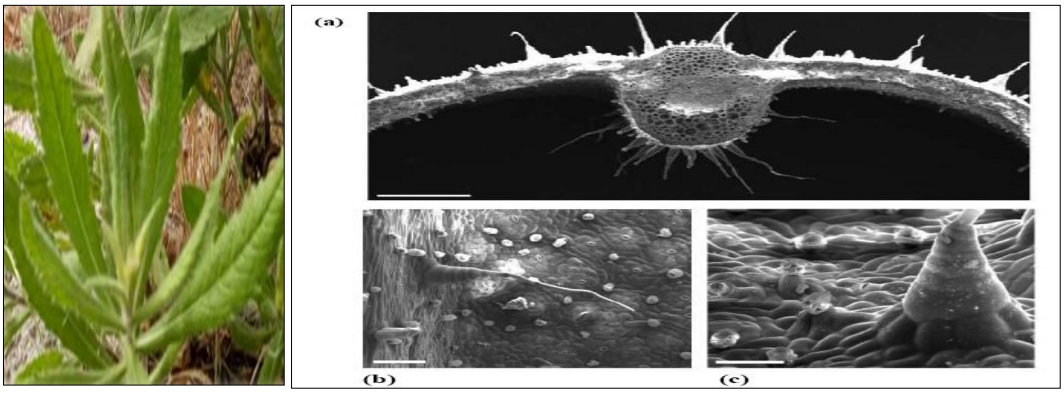


Fig.1. (a) SEM view of a cross-section of the Inula viscosa sheet x 500μm. (b), (c) SEM view of surface (b) x 100μm, (c) x 50μm

2.2 Reagents and standards

Aluminum chloride, ascorbic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ethanol, folinciocalteu phenol reagent, gallic acid, methanol and quercetin were purchased from Sigma-Aldrich-Riedel de Haën (Sigma–Aldrich GmbH, Sternheim, Germany). Hydrogen chloride and xylene from Cheminova (CheminovaInternacional S.A., Madrid, Spain). Ethyl acetate from Prolabo (Merck) (Paris, France)

2.3. Extraction procedure

The aqueous extraction of the polyphenols from the inula viscosa was carried out batchwise in a 2L reactor equipped with a powerful propeller stirrer (Model RW Basic 28, IKA, Artur Nogueira, SP, Brazil), the anchor stirring has been used because it is ideal for media of medium or high viscosity. The volume of extraction water was 1.5 L. The extractions were carried out under different operating conditions of temperature, solid to liquid ratio and stirring speed mentioned in Table 3. The extracts obtained are then filtered and analyzed for the determination of the content of total polyphenols, flavonoids and anthocyanins and of the antiradical activity.

2.4. Analytical methodology

2.4.1 Determination of total polyphenols (TP)

The total content of phenolic compounds was determined by UV-VIS spectrophotometry (Secomam S.250 UV/VIS Spectrophotometer) according to the Folin-Ciocalteu method described by [11] , calculated from a calibration curve (Abs=0.00935×C+0.098 , R2=0.998), where C [mg GAE.mL^{-1}] and expressed as milligram gallic acid equivalent per g dry weight material (mg GAE. g^{-1} DW).

2.4.2 Determination of flavonoids (FLA)

The flavonoid content was determined by a colorimetric assay using a method described by [12]. The concentration of flavonoids were deduced from a standard curve (y= 0.035 x+ 0.288, R2= 0.995), where C is the concentration of quercetin (µg.mL^{-1}) and calculated in mg quercetin equivalent (QE) per g dry weight material (DW).

2.4.3 Determination of anthocyanins (AN)

The anthocyanins (AN) content of the aqueous extracts was determined using the method described by [13],calculated using the following equation proposed by Di Stefano et al,1989: AN = 16.17.ABS540 .D, where D is the dilution factor, and expressed as milligrams of malvidin equivalent per gram of dry weight material (mg ME. g^{-1} DW).

2.4.4 Determination of antiradical capacity (DPPH radical scavenging activity)

Extracts antiradical activity was estimated using DPPH° radical scavenging ability method according to the procedure described by [14,15]. The antiradical value represents the percent of radical scavenging ability according to the percentage inhibition as calculated by the flowing equation.

% inhibition = [(Abs bk – Abs s) / Abs bk] * 100, where: Abs bk: blank absorbance, Abs s: sample absorbance,

The plot of percentage inhibition versus different concentrations of antioxidants gives the inhibitory concentration IC50 (µg.mL^{-1}) which corresponds to a 50% reduction of the activity of DPPH in the reaction medium.

The DPPH° concentration in the reaction medium was calculated from the following calibration curve determined by linear regression:

Abs 517 nm = 0.0257 * C DPPH , R^2= 0.9987, where C [µg.mL^{-1}].

The percentage of the remaining DPPH° against standard concentration was calculated as follows: % DPPH° REM = 100 x $\frac{[DPPH^0]_t}{[DPPH^0]_{t=0}}$ (where [DPPH°]t=0 and [DPPH°]t are concentrations of DPPH° at t= 0 and t= t respectively) and plotted as a function of different

ratios (Rm) of the amount of antioxidant to that of initial DPPH to obtain the amount of antioxidant necessary to decrease the initial DPPH° concentration by 50% (Effective concentration, EC50 ,expressed in mg of antioxidant (AO) per g of DPPH°) and the time needed to reach the steady state to EC50 concentration (TEC50).

The Antiradical efficiency (AE) was calculated according to Sanchez Moreno [16] as follows:

$AE = \frac{1}{EC50 * TEC50}$ [g DPPH°. (mgAO.min)$^{-1}$]

According to [15], samples were divided into four antiradical efficiency groups:

AE < 1. 10^{-3} : Low antiradical activity

1.10^{-3} < AE < 5. 10^{-3} : medium antiradical activity

5. 10^{-3} < AE < 10. 10^{-3} : high antiradical activity

AE > 10. 10^{-3} : Very high antiradical activity

2.4 Statistical analysis

To study the influence of temperature (X1), S / L ratio (X2) and stirring rate (X3) on phenolic content and antioxidant characteristics of aqueous extract of Inula viscosa the experimental design method was implemented for two plant lots.

Preliminary tests have determined the time required to reach extraction equilibrium which is 2 hours [17].

The optimization of these three operating parameters is carried out by means of an orthogonal composite plane with three independent variables. The three independent variables were chosen and coded at five levels (-α, -1, 0, +1, +α) which resulted in an experimental design of 18 experimental points, including four central points

The equations giving the variations of the content of phenolic compounds, flavonoids, anthocyanins and the antiradical activity as a function of the extraction conditions were determined. The values of the operating factors optimizing the different responses have been established.

A multi-response optimization has made it possible to determine the conditions that maximize both the quantity of polyphenols and the antiradical power. The Statgraphics (Centurion 17.1.02) software was used

The tests and the analysis for the characterization of the recovered extracts were carried out three (03) times. The mathematical models were validated by carrying out two experiments included in the field of study but different from the 18 experiments carried out.

3. RESULTS AND DISCUSSIONS

Table 1. Response surface design and corresponding response values for aqueous extraction

Process variables real and coded values			TP (mgME. g^{-1} DW)		FLA (mgQE. g^{-1} DW)		AN (mgME. g^{-1} DW)		DPPH (%) % inhibition	
X1 (°C(-))	X2 (g.L-1 (-))	X3 (rpm) (-))	July	December	July	December	July	December	July	December
32(-1)	10(-1)	300(+1)	132	135	7,02	5,35	0,76	0,71	75	73
32(-1)	10(-1)	100(-1)	128	129	8,29	6,62	0,67	0,66	75	73
42(0)	58(+1,414)	341,4(0)	105	97	7,32	5,65	0,12	0,10	75	74
52(+1)	10(-1)	100(-1)	244	194	17,49	15,82	0,46	0,44	81	79
42(0)	30(0)	341,4(+1,414)	134	108	8,04	6,37	0,27	0,25	81	79
42(0)	30(0)	58,6(-1,414)	113	102	6,36	4,69	0,21	0,19	78	76
27,86(-1,414)	30(0)	200(0)	97	80	5,64	3,97	0,13	0,11	71	71
42(0)	2(-1,414)	200(0)	284	246	18,92	17,25	0,71	0,67	82	81
32(-1)	50(+1)	300(+1)	114	94	5,17	4,84	0,15	0,14	73	72
56,14(+1,414)	30(0)	200(0)	129	120	8,85	7,18	0,11	0,09	81	80
52(+1)	10(-1)	300(+1)	245	195	15,32	13,65	0,57	0,54	82	81
52(+1)	50(+1)	100(-1)	115	90	5,60	5,27	0,13	0,11	76	74
52(+1)	50(+1)	300(+1)	118	100	6,62	6,29	0,18	0,16	76	74
32(-1)	50(+1)	100(-1)	106	92	5,45	5,22	0,14	0,12	73	72
42(0)	30(0)	200(0)	125	108	5,54	5,12	0,29	0,27	80	80
42(0)	30(0)	200(0)	125	107	5,72	5,05	0,27	0,26	80	80
42(0)	30(0)	200(0)	125	108	5,64	5,13	0,28	0,26	81	80
42(0)	30(0)	200(0)	126	108	5,54	5,08	0,30	0,27	80	80

Table 2. Polynomial equations describing experimental data of phenolic compounds content and antiradical activity of Inula viscosa aqueous extracts and their respective regression coefficients R^2

Response	Model equation	R^2
Total polyphenols July mg GAE. g^{-1} DW	TP = 125,8 + 24,1X1 -45,9 X2 +33,6 $X2^2$ -1,8 $X3^2$ -27 X1X2	0,94
Total polyphenols December mg GAE. g^{-1} DW	TP = 119,5 + 30,9X1 -81,3 X2 +52,3 $X2^2$ -30,3 X1X2	0,97
FLA (mg QE. g DW^{-1}) July	FLA = 8,83 + 38,3 X1- 7,1X2 - 3,8 X1X2	0,96
FLA (mg QE. g DW^{-1}) December	FLA = 6,79 + 1,96 X1- 3,0 X2 - 2 X1X2	0,94
AN (mg ME. g DW^{-1}) July	AN= 0,01 - 0,03 X1 - 0,22 X2 +0,2 $X2^2$ + 0,11 $X3^2$ +0,05 X1X2	0,99
AN (mg ME. g DW^{-1}) December	AN= -0,014 -0,068 X1 – 0,43 X2 + 0,39 $X2^2$ + 0,23$X3^2$ + 0,11 X1X2	0,99
Antiradical activity DPPH (%) July	DPPH = 81+2,72X1-1,99X2-2,75$X1^2$-X1X2	0,98
Antiradical activity DPPH (%) December	DPPH = 81+5,12X1-3,98X2-5,49$X1^2$-2,5X1X2	0,96

X1: Temperature, X2: solid to liquid ratio, X3: Stirring speed: Coded values.

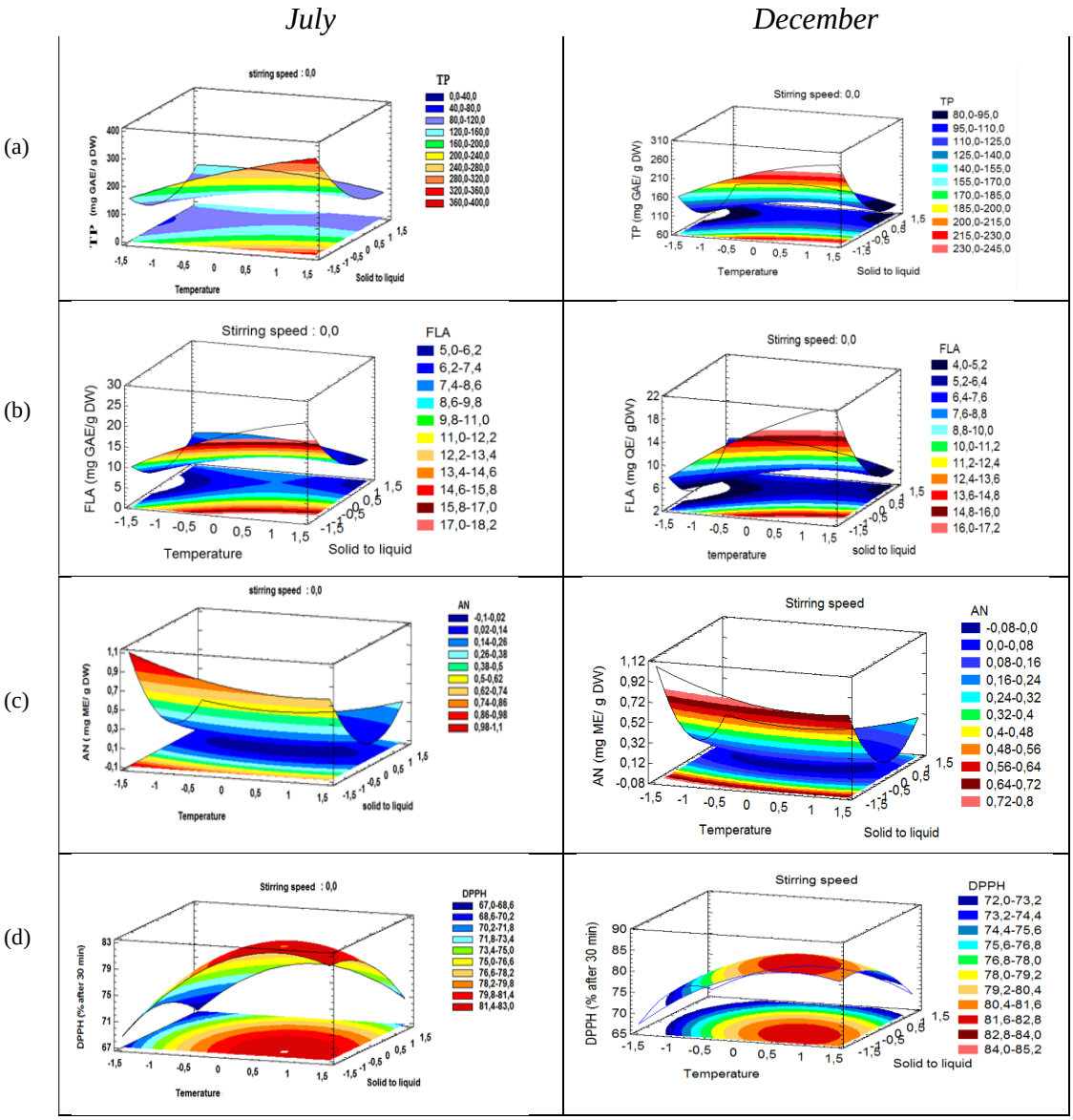


Fig.2. Response surfaces plot showing the interaction effect of temperature and solid to liquid on the level of polyphenols (a) , flavonoids content (b), yield of anthocyanins (c) and antiradical activity as mesuread by DPPH(d)of Inula viscosa aquous extracts. The variables are presented in their coded levels and the stirring speed is held at her central value

Table 3. Values of the operating parameters giving the optimum in phenolic compounds and antiradical power for the two lots studied

Response	**Operating parameters giving the optimum**			**Optimal value**
	Temperature (°C)	S/L ratio ($g.L^{-1}$)	Stirring speed (rpm)	
Total polyphenols July mg GAE. g^{-1} DW	56(+1,41)	2(-1,41)	253(0,53)	33,26
Total polyphenols December mg GAE. g^{-1} DW	56(+1,41)	2(-1,41)	211(0,11)	26,18
FLA (mg QE. g DW^{-1}) July	56(+1,41)	2(-1,41)	146(-0,54)	23,08
FLA (mg QE. g DW^{-1}) December	56(+1,41)	2(-1,41)	164(-0,36)	21,45
AN (mg ME. g DW^{-1}) July	28(-1,41)	2,8(-1,36)	341(+1,41)	1,24
AN (mg ME. g DW^{-1}) December	28(-1,41)	-1,36 2,8	341(+1,41)	1,18
Antiradical activity DPPH (%) July	48,9 (0,69)	7,8(-1,11)	237(0,37)	83,11
Antiradical activity DPPH (%) December	48,6 (0,66)	-13,4(-0,83)	225(0,25)	82,73

X1 : Temperature, X2 : Solid to liquid ratio, X3 : Stirring speed : coded value.

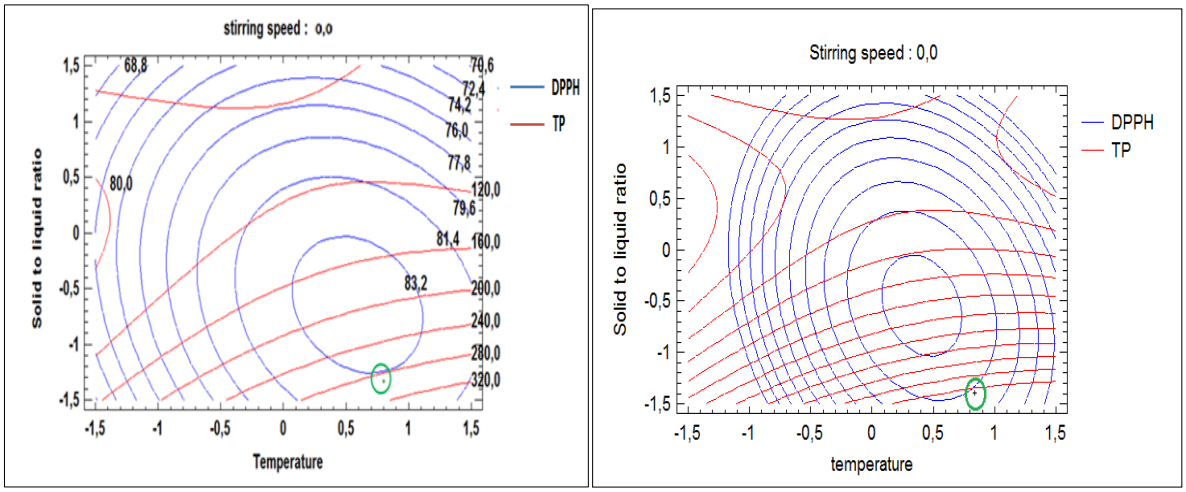
The influence of the technological parameters studied on the different responses is the same for the two months of July and December. The estimated response surfaces, given in Fig 2, show that the yield of polyphenols, flavonoids and the antiradical activity increase with

increasing temperature. Nevertheless it has a negative effect on the anthocyanin content. The negative influence of the solid to liquid ratio on the extraction rate of the different groups of polyphenols as well as on the antiradical power is important. The stirring speed slightly affects the extraction yield with a positive effect. All interactions are insignificant with the exception of the interaction between temperature and solid to liquid ratio. This is due to several factors [17]. However, the levels of the various phenolic compounds in the July batch are relatively higher than in December. A similar result is recorded for the antiradical activity. The antioxidant characteristics calculated for the month of July are comparatively higher than the values found for the month of December. Similarly, the values giving the optimum for the various responses show a difference between the two lots studied. Indeed according to the studies of [18] , the inula viscosa begins to produce new leaves at the end of March. In mid-May the new leaves reach their full expansion. Subsequently, in the late summer and early autumn, new leaves are continuously produced by the plants and until the end of October. From November, only a few leaves are green and most plants turn grayish because of the strong leaf senescence. In mid-November, only 5% of the leaves of the plants are green, the rest are dry and supposedly dead. This condition is maintained throughout the entire winter until the end of March (northern hemisphere spring) when new leaves are sprouted. Indeed the first picking of the plant was made in early July. According to [19] during the main growth period (March - June), there is accumulation in all parts of the plant of secondary metabolites where the maximum is reached in June and then it decreases more and more to the senescence of the leaves. The content of phenolic compounds in December, which presents in our study the end of the previous vegetative cycle is lower compared to the month of July. [20] described in their study that the potential of the substance content stored in the mature leaves of the inula viscosa decreases continuously after flowering until old age and leaf defoliation.

3.3 Determination of the optimum extraction conditions

Fig 2 shows that factors values giving the optimum of different responses are not the same. Nevertheless, it would be very useful to determine the optimum conditions of both functions, yield of polyphenols and antiradical activity. We proceed to a multiple response optimization using Statgraphics (Centurion 17.1.02). It determines the parameters of experimental factors giving the desired characteristics for response variables simultaneously. This is accomplished by constructing a desirability function, based on the values of response variables, which is then optimized. The optimal point surrounded by the green circle in the graphic of overlaying the contours of the two responses correspond to a temperature of (50°C, 51°C), a solid to

liquid ratio of (3 g. L^{-1},2 g.L^{-1}) and a stirring speed of (217 trs.min^{-1},220 trs.min^{-1}) for the months of July and December respectively. Under these extraction conditions, the intended optimum has a content of phenolic compounds of (291 et 251 mg GAE. g DW^{-1} respectively) and an antiradical activity of 83% (for the two lots) close to the average values found by the experimental results.



(a) (b)

Fig.3. Superposition of contour charts for total polyphenols content (TP) and antiradical activity (DPPH) showing the optimal zone (green circle) for the two considered factors

3.4. Determination of the antioxidant characteristics of the optimum extraction of the two lots

In order to determine the antioxidant characteristics of the extracts recovered under the optimal conditions of extraction (July and December), we carry out a purification in liquid phase. Three successive washes of the aqueous phase with the same volume of ethyl acetate were carried out. 92% of phenolic compounds were recovered for the month of July against 93% for the month of December. A Vacuum distillation using a Büchi R-210 rotavapor (BÜCHI Labortechnik AG) was used to recover the purified extract. A measurement of the total polyphenol concentration and the antiradical activity as well as a determination of the antioxidant characteristics of the two batches was made. The results are given below:

(a) (b)

Fig.4. *Purification of raw extracts: Liquid-liquid extraction (a) , Vaccum distillation (b)*

3.4.1 Determination of inhibitory concentration (IC50)

The evolution of the percentage inhibition (I%) is plotted as a function of the concentration of the sample. For I = 50%, the IC50 is determined graphically. It is expressed in (µg / ml)

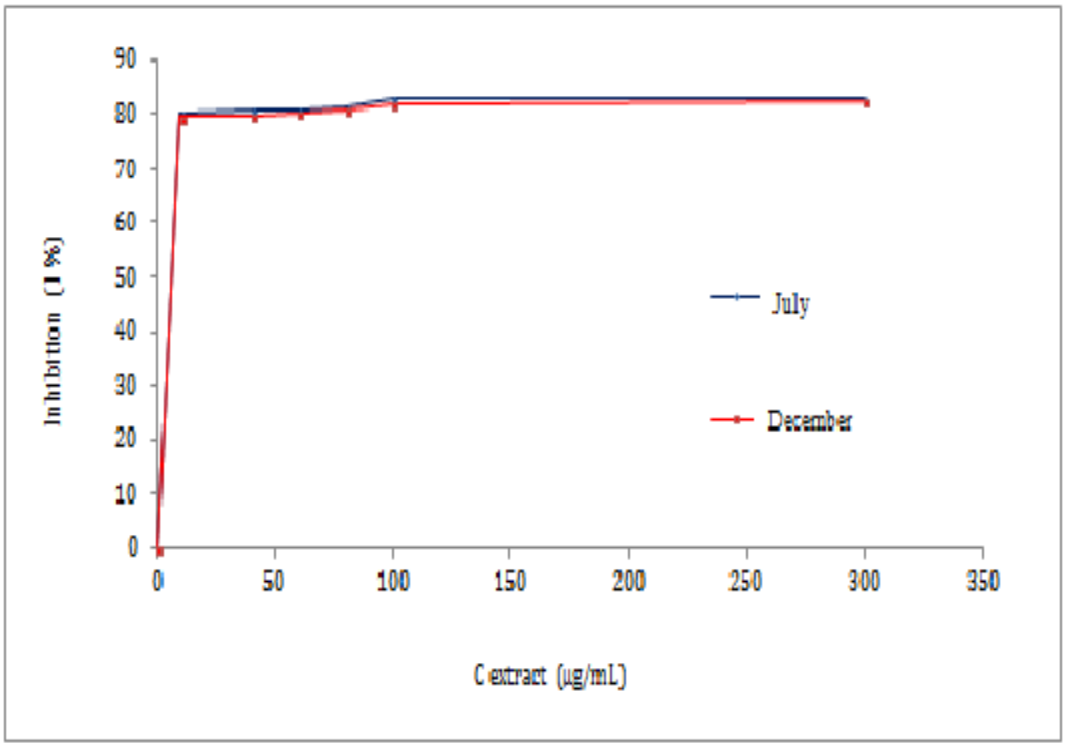


Fig.5. Variation of percentage inhibition (% I) as a function of the concentration of the extract

3.4.2 Determination of the effective concentration EC50

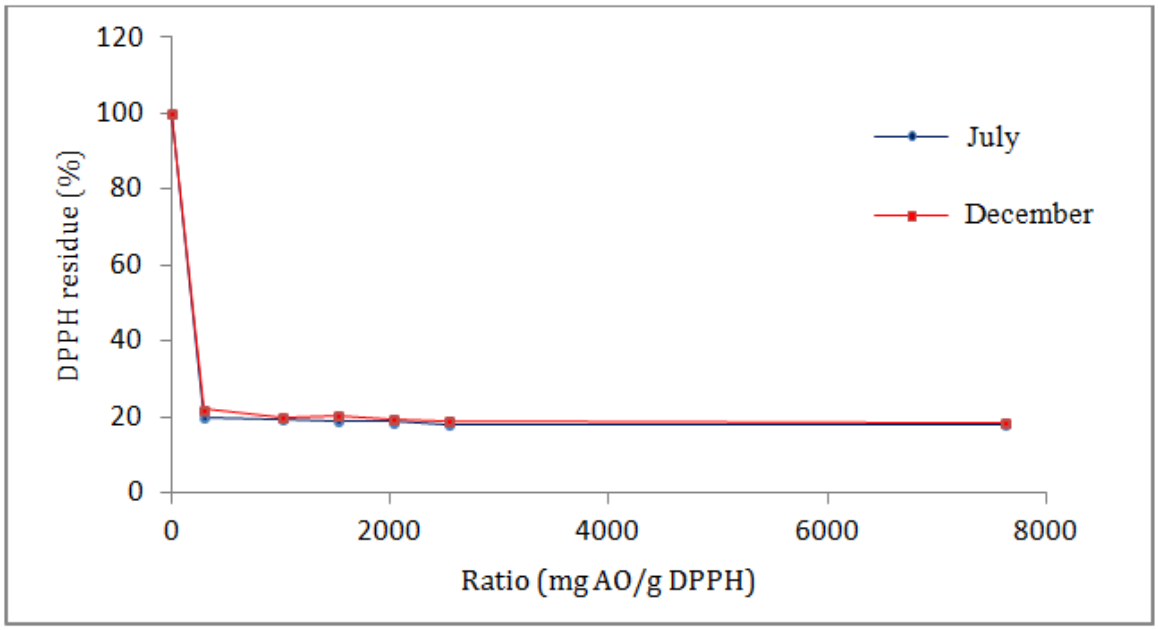


Fig.6 . DPPH residue variation (%) versus ratio (mg AO / g DPPH)

3.4.3. Determination of TCE50 time

Table 4. Evolution of antiradical activity of inula viscosa extracts as a function of time

Time (min)	1	5	10	15	20	25	30	32	40	50	60
Antiradical activity (%) July	38,1	42,8	50.1	53,8	56,2	57,9	58,2	58,2	58,2	58,2	58,2
Antiradical activity (%) December	36,2	40,7	49.9	52,1	55,6	56,5	57,3	57,6	57.9	57.9	57.9

Table 5. Antioxidant characteristics of purified extracts under optimal extraction conditions

Lot	Essay	Operating Parameters			Responses (Purified extract)					
		T (°C)	S/L ($g.L^{-1}$)	ω (rpm)	polyphenols yield (mg GAE. g^{-1} DW)	DPPH (%) inhibition	IC50 ($\mu g.mL^{-1}$)	EC50 (mg AO.(g $DDPH^{\circ})^{-1}$)	TEC50 (min)	AE (g DPPH° . (mg AO. $mn)^{-1}$)
July	1	50	3	217	267	90	5,0	125	8,0	$1,00.10^{-3}$
	2	50	3	217	265	90	4,8	120	7,8	$1,10.\ 10^{-3}$
	3	50	3	217	272	90	5,2	130	8,2	$0,94.\ 10^{-3}$
	moy	50	3	217	268	90	5,0	125	8,0	$1,00.\ 10^{-3}$
December	1	51	2	220	246	89	6,5	129	10,0	$0,77.\ 10^{-3}$
	2	51	2	220	252	89	7,1	134	9,8	$0,76.\ 10^{-3}$
	3	51	2	220	248	88	6,8	136	9,5	$0,77.\ 10^{-3}$
	mean	51	2	220	249	89	6,8	133	9,8	$0,77.\ 10^{-3}$

With an antiradical efficiency index 1.10^{-3} <AE <5.10^{-3} g DPPH ° (mg AO min) $^{-1}$, the extract recovered in July belongs to the group of antioxidants with intermediate antiradical activity. While the extract of the month of December is classified in the group of antioxidants with low anti-radical activity AE <1.10^{-3} g DPPH ° (mg AO min) $^{-1}$

4. CONCLUSION

The use of a green solvent namely water at moderate temperature (T <56 ° C) gave high levels of phenolic compounds with a high antiradical power. The period of collection of the plant influences on the amount of phenolic compounds recovered, on the antiradical power, on the antioxidant characteristics and on the values of the operating conditions optimizing the extraction. The multi-response optimization of the extraction conditions (T = 50 ° C., S / L = 3 g. L^{-1} and w = 217 $tr.min^{-1}$ and (T = 51 ° C., S / L = 2 g. L-1 and Vag = 220 tr.min-1) for the months of July and December respectively indicate a richness in phenolic compounds (291 and 251 mg EAG.gMS-1) for the months of July and December respectively. The purified inula viscosa extracts have high antiradical activities (90 and 89% for the month of July and December respectively) comparable to the characteristics of powerful antioxidants (gallic acid and ascorbic acid). The qualitative analysis of the two extracts obtained remains of crucial interest in order to be able to choose the operating conditions and the collection period giving the maximum product desired.

5. REFERENCES

[1] Miguel M G. Antioxidant and Anti-Inflammatory Activities of Essential Oils: A Short Review. Molecules ,2010, 15, 9252-9287

[2] Mehinagic E, Bourles E, Jourjon F . Composés des fruits d'intérêt nutritionnel: impact des procédés de transformation sur les polyphénols . Revue suisse Viticulture, Arboriculture, Horticulture. 2011 , 43 , 6, 364–368.

[3] Harborne J B , Williams C A. Advances in flavonoid research since 1992 . Phytochemistry, 2000, 55 , 481-504

[4] Abu Zarga M H, Hamed E M, Sabri S S, Oelter W, Zeller K P. New sesquiterpenoids from the Jordanian medicinal plant Inulaviscosa. J.Nat Prod, 1998, 798-800.

[5] Danino O, Gottlieb E H, Grossman S, Bergman M. Antioxidant activity of 1,3-dicaffeoylquinic acid isolated from Inula viscose. Food Res Int, 2009, 42 ,1273-1280.

[6] Wollenweber E, Mayer K, Roitman J N. Exudate flavonoids of Inula viscosa, Phytochem, 1991, 2445–2446.

[7] Andolfi A, Zermane N, Cimmino A, Avolio F, Boari A, Vurro M, Evidente A, Inuloxins A-D, phytotoxic bi-and tri-cyclic sesquiterpene lactones produced by Inulaviscosa: potential for broomrapes and field dodder management. Phytochemistry, 2013, 86, 112-120.

[8] Haoui I E, Derriche R, Madani L, Oukali Z. Analysis of the chemical composition of essential oil from Algerian Inula viscosa (L.) Aiton. Arabian J. Chem, 8, 2011, 587-590.

[9] Sanz J F, Ferrand C , Alberto Marco J. Oxygenated nerolidol esters and eudesmane acids from Inula viscose. Phytochemistry, 1991, 30, 3653-3655.

[10] Grande M, Torres P, Piera F , Bellido I S. triterpenoids from dittrichia viscosa. Phytochemistry, 1992, 31, 1826-1828.

[11] H.B. Li , K.W. Cheng, C.C. Wong , K.W. Fan , F.Chen, Y. Jiang, Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem, 2007, 102, 71–776.

[12] Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P , Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry, 2006, 97, 654-660.

[13] Di Stefano R, Cravero M C, Genfilini N.Metodi per lo studio dei polifenoli dei vini, L'Enotecnico, 1989, 5 , 83-89.

[14] Que F,Mao L, X.Pan, Antioxidant activities of five Chinese rice wines and the involvement of phenolic compounds. Food Res Int, 2006, 1, 581-587.

[15] Boubekri C, Lanez T, Djouadi D. A comparative study on antioxidant activities and phenolic contents of five Algerian eggplant cultivars, St. Cerc. St. CICBIA **2015** 16 (1), 029-036

[16] Scherer R, Godoy H T. Antioxidant activity index (AAI) by the 2,2 diphenyl-1-picrylhydrazyl method, Food chem,2009, 112, 654-658.

[17] Amrouche S , Derriche R , Messaoudi S , Lahouazi N . Optimization of Phenolic Compounds Extraction from Algerian Inula viscosa (L.) Aiton leaves, 2016, Mor. J. Chem, 2016, 4, 1117-1129

[18] Parolin P, Scotta M I, Cécile Bresch C. Notes on the phenology of Dittrichia viscose. Journal of Mediterranean Ecology , *2013* , 12, 27-35

[19] Quézel, P, Santa, S. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Ed. CNRS ,Paris, 1963.

[20] Reverchon, E, De Marco, I. Supercritical fluid extraction and fractionation of natural matter. Journal of Supercritical fluids, 2006, 38, 146-166.

How to cite this article:
Amrouche S, Mohellebi F, Derriche R. Extraction of antioxidant phenolic compounds from Algerian inula viscosa leaves: influence of the harvest period and optimization. J. Fundam. Appl. Sci., 2019, *11(3), 1212-1227.*