Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
H. Hazhazi/ Mor. J. Chem. 4N°4 (2016) 1117-1129

Optimization of Phenolic Compounds Extraction from Algerian Inulaviscosa (L.) Aitonleaves

Sara Amrouche *, RatibaDerriche, Saida Messaoudi , Naima Lahouazi

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

Abstract

Extraction of polyphenols from Inulaviscosa (L.) leaves has been studied using water as a green solvent. Optimization of the process was performed using response surface methodology (RSM) in order to investigate the effects of three operating parameters, temperature, solid to liquid ratio and stirring speed (ω) in the following ranges : (28-56 ° C), (2-58 g.L-1) and (60-341 rpm), respectively. The efficiency of extraction was assessed by measuring polyphenols, flavonoids, and anthocyanins contents as well as antiradical activity. The conditions optimizing the yield of polyphenols and antiradical activity were established (T=50°C, S/L= 3 g.L-1, ω= 217 rpm). Under these conditions, polyphenols content was estimated to 291 mg GAE .g DW-1, and antiradical power was 83%. Purification of the extract obtained under these optimal extraction conditions increased the antiradical activity to 90%. This antiradical activity is medium (Antiradical efficiency = 10-3 g DPPH°. (mg AO. min)-1) with antioxidant characteristics (IC50= 5µg.mL-1, EC50 = 125 mg AO. (g DPPH°)-1 and TEC50 = 8min) comparable to those of powerful antioxidants. These results are very promising especially as antioxidant phenolic compounds have beneficial effects on health, and can find major applications in food and pharmaceutical products.

.

** Corresponding author:*
sara.amrouche@g.enp.edu.dz
Received 20 Sept 2016,
Revised 16 Dec 2016,
Accepted 16 Jan 2017

Keywords: *Inulaviscosa (L.) , Phenolic compounds , Antiradical activity , Extraction , Green solvent, Optimization*

1. Introduction

Inulaviscosa (L.) Aiton [Synonym : Dittrichiaviscosa (L.) Greuter][1] is a perennial plant of the Asteraceae family, widely distributed in the Mediterranean area. It has a very diverse therapeutic history and used since ancient times in the traditional medicine. In Algeria, this plant has been used to treat bronchitis, cough, tuberculosis, anemia, malaria and the urinary tract diseases, for the treatment of stomach pathologies, arterial hypertension, diabetes, sunburn, hemorrhoids, and as a poultice for rheumatic pain [2]. Because of its high therapeutic potential and high abundance, this ruderal caught recently, the attention of many researchers who have proved by their results, the data of traditional medicine [3,4]. They have also highlighted its antioxidant properties and high content in phenolic compounds [5,6]mainly composed of flavonoids [7],sesquiterpene acids [8,9,6] and triterpene esters [10,11].However, there is no published work studying the influence of various operating conditions on the phenolic content and radical scavenging activity of Algerian inulaviscosa extracts. Polyphenols are becoming increasingly famous due to their numerous reported beneficial effects on health :Protective action against different types of cancer[12,13] against many skin disorders [14] degenerative and neurodegenerative diseases [15]. Other applications are encountered in food and cosmetics industries, mainly due to their antioxidant properties [16]. Recently, new data indicate that synthetic antioxidants used in the industry could have carcinogenic effects on human. This focused the attention of researchers on the extraction of natural polyphenols with proved antioxidant properties. The solid-liquid extraction using organic solvents was the most used method for the recovery of polyphenols, the choice of the adequate solvent being critical in the food and pharmaceutical industries. As a result, many authors have developed new extraction methods using environment friendly solvents.Indeed, water is at the top of the EHS classification scale. It is a preferable solvent according to Pfizer traffic light[17]. In the context of sustainable development, we choose water as the extraction solvent.Based on these considerations, the present study represents the first attempt conducted with a primary objective to optimize the aqueous extraction of polyphenols from Algerian Inulaviscosa in a batch system. At first, the study investigated the effect of the nature of the process and the solvent used on yield and antiradical power of the recovered extract. The influence of three key operating parameters of the solid-liquid extraction of plant substrates (Temperature , solid to liquid ratio and stirring speed) on polyphenols, flavonoids, and anthocyanins contents as well as on antiradical activity was also assessed. The conditions optimizing the yield of polyphenols and antiradical activity simultaneously were established using an experimental design method. A purification of the extract obtained under optimum extraction conditions was then achieved and his antioxidant characteristics were determined and compared to those of powerful antioxidants (Gallic acid and ascorbic acid).

2. Materials and methods

2.1. 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)

2.2. Plant material

The Inulaviscosa samples were collected in July 2014 in Ben Aknoun, 6 km southwest of Algiers (Algeria), at 270 meters elevation. The leaves used were dried in a ventilated dark room to increase the life span and inhibit enzymatic degradation and bacterial growth. The leaves are crushed to an average particle size of 237µm and have a moisture content (H) of 2% , packed in a sealed container and stored at 5 ◦C until required.

2.3. Experimental design

The water extraction of polyphenols from Inulaviscosa was carried out batchwise in a 2L reactor equipped with a powerful propeller stirrer (Model RW 28 Basic , IKA, ArturNogueira, SP, Brazil), the anchor stirring was used because ideal for medium to highly viscous fluids. The volume of water in the extraction flask was 1500 mL. Extractions were performed under different operating conditions of temperature, solid to liquid ratio and stirring speed (Table 3). The extracts obtained were filtered and analyzed for the determination of polyphenols, flavonoids and anthocyanins contents. The antiradical activity was also determined.

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 [18] , calculated from a calibration curve ($Abs=0.00935\times C+0.098$, $R^2=0.998$), where C [µgGAE.mL^{-1}] and expressed as milligram gallic acid equivalent per g dry weight material (mg GAE. g DW^{-1}).

2.4.2 Determination of flavonoids (FLA)

The flavonoid content was determined by a colorimetric assay using a method described by [19]. The concentration of flavonoids were deduced from a standard curve ($y= 0.035\ x+ 0.288$, $R^2= 0.995$), 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 [20],calculated using the following equation proposed by Di Stefano et al,1989: $AN = 16.17.ABS_{540}.D$, where D is the dilution factor, and expressed as milligrams of malvidin equivalent per gram of dry weight material (mgME.gDW^{-1}).

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 [21]. 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: $\%\ DPPH°_{REM} = 100 \times \frac{[DPPH°]t}{[DPPH°]t=0}$ (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 [22] as follows:

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

According to [22], 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

2.5. Statistical analyses

An optimization of three key operating parameters of the solid-liquid extraction of plant substrates using an experimental design method has been achieved. This study objective was to investigate the influence of temperature (X1), solid to liquid ratio (X2) and stirring speed (X3) on extraction yield of phenolic compounds, flavonoids, anthocyanins and antiradical activity of Inulaviscosa extracts. Preliminary tests have determined the time required to reach extraction equilibrium which is 4 hours. At t=2 h, the polyphenols yield is estimated at 97% of the maximum yield. Thus for the remainder of experiments the extraction time was fixed at 120 minutes. A composite orthogonal design with three independent variables was used. 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. Statistical significance of the variables was determined at the 5% probability level ($p < 0.05$). The data obtained were fitted to second order polynomial equations and where possible the models were simplified by elimination of statistically insignificant terms. The Statgraphics (Centurion 17.1.02) software (trial version) was used. Assays to validate the optimum extraction conditions as well as the analysis for characterization of the obtained extracts were performed in triplicate.

3. Results and discussion

3.1 Preliminary results

The first extraction tests on Inulaviscosa leaves were performed in a soxhlet under the operating conditions (H = 15%, S / L = 20 g. L^{-1} and t = 8 h) and showed decreasing yields depending on the polarity of the solvent used. The same observation stands for the antiradical activity.

Table 1 :Total polyphenol content and antiradical activity represented by IC50 (measured by the DPPH test) of Inulaviscosa extracts

Extraction solvant		Recoveredextract	
	Dielectric constant (25°C)	Total polyphenol (mg GAE.g DW^{-1})	IC50 (µg. mL^{-1})
Methanol	32.6	106.34 ± 1.49	5.33 ± 0.58
Ethanol	24.5	70.79 ± 1.20	17.67 ± 2.08
EthylAcetate	6.0	64.32 ± 1.28	22.00 ± 2.00
Acetone	20.7	51.25 ± 0.71	30.00 ± 5.00
Hexane	1.9	17.03 ± 0.43	790.00 ± 10.00

The recovery tests of Inulaviscosa essential oils using three methods (Steam distillation, Hydrodistillation, Hydrodiffusion) under the following operating conditions (H= 21 % , m=20 g , q_v= 12 mL.min^{-1}and t= 5h) also showed that the essential oils even when their yields are low, presented very valuable antiradical activity. The hydrosols obtained from these three processes reveal the richness in phenolic compounds with important antiradical activity.

Table 2 :Total polyphenol content and antiradical activity represented by IC50 (measured by the DPPH test) of Inulaviscosa hydrosols

Hydrosol	Total polyphenols (mg GAE. g DW^{-1})	IC50 (µg. mL^{-1})
Steam distillation	291.02 ± 33.42	0.33 ± 0.11
Hydrodistillation	388.00 ± 40.56	4.50 ± 0.50
Hydrodiffusion	314.71 ± 92.29	0.90 ± 0.10

These preliminary results highlight the influence of the nature of the process and the solvent used on yield and antiradical power of the recovered extract. In all cases the use of water gives the best results in terms of polyphenols yield and antiradical activity. So we focused our work towards the use of less energy intensive process using a green solvent namely: Water extraction. We then proceeded to optimize the operating conditions and we also evaluated the levels of flavonoids and anthocyanins.

3.2 Optimization of extraction process

Table 3 shows the actual and coded values assigned to each factor as well as the results of experiments for all the studied responses. The maximum polyphenols yield obtained was 284 mg GAE. g DW^{-1} (assay 8, Table 3). This important value is comparable with a reported values for some sources rich in phenolic compounds like groundnut seeds (93-230 mg GAE.g DW^{-1})[23], apple pomace (43-1711 mg GAE.g DW^{-1}) [24]and chestnut wood (102-209 mg GAE.g DW^{-1}) [25].As for the results of Inulaviscosa extraction , [5,26] report similar yields of total polyphenols (274 mg GAE. g DW^{-1} and 314 mg GAE .g DW^{-1} respectively) , during the extraction of the plant with ethanol and methanol respectively. However, these two studies found a flavonoids content (44.8and 48.3 mg QE. g DW^{-1} respectively) higher than the values obtained in our study (5.17- 18.92 mg QE. g DW^{-1}, Table 3). As regards anthocyanins, an important amount was recovered (0.76 mg ME . g DW^{-1}, Table 3) higher than the values found by [28] during the extraction of polyphenols from Vitexagnus-castus L. (0.38 -0.60 mg ME. g DW^{-1}). A considerable antiradical power defined by the percentage of inhibition of DPPH$^{\circ}$ radical was also recorded for all aqueous extracts (71-82%, Table 3). These values are similar to those reported by some authors [24]. The determined inhibitory concentrations (IC50) (varying from 10 to 16 µg AO. mL^{-1}, Table 5) are lower than the values obtained in other studies [5,28]. They are nevertheless greater than the values determined for some powerful antioxidants [Gallic acid: 3µg.mL^{-1}, Ascorbic acid: 8µg.mL^{-1}, Table 5]. The antiradical efficiency index of all extracts was lower than 1.10^{-3} g DPPH°. (mg AO.mn)$^{-1}$, therefore, the aqueous Inulaviscosa extracts belong to the group of antioxidants with low antiradical activity. The influence of various factors on the extraction results is given in Fig.1. The response surfaces repesented in (Fig. 2) show that the yield of polyphenols, the flavonoids content and the antiradical activity increase with increasing temperature. This positive effect is more important for low solid to liquid ratios (For 10 g.L^{-1}and a

stirring speed of 300 rpm, on passing from a temperature of 32 °C to 52 °C, the increase in yield is 87% and 155% for the polyphenols and flavonoids respectively, while the antiradical power is 9% higher).This is due to the fact that heat facilitates extraction by permeabilizing the cell wall , increasing the solubility of the material to be removed as well as the diffusion coefficient and finally by reducing the viscosity of the extraction juice [29]. For temperatures below 60°C , a similar effect of temperature on the extraction yield of polyphenols and on the antiradical potential of different plant substrates was observed [30-33]. However for the same range of temperature, [34] found a negative effect of temperature on the aqueous extraction yield of phenolic compounds of pequi (CaryocarbrasilienseCamb). Furthermore, other works have shown that temperature present a positive effect on polyphenols yield within a certain range of temperature. On the other hand, an excessive temperature denatures the products to be extracted [35, 25] Indeed, the polyphenols content decreases, because inactive compounds will be extracted [36]. As for anthocyanins, a detrimental effect of temperature on the extraction yield was observed. This may be due to the fact that anthocyanins are highly unstable molecules and easily prone to degradation by various factors such as light, pH, temperature, presence of sulfite, ascorbic acid or enzymes, among others [37].Some studies[38] showed that an increase in temperature is associated with a substantial degree in anthocyanins destruction.

Table 3: Response surface design and corresponding response values for aqueous extraction.

Order of experiences	Process variables real and coded values			Composition			% inhibition (I %)
	X1 (°C(-))	X2 ($g.L^{-1}$(-))	X3 (rpm(-))	TP (mgGAE.gDW^{-1})	FLA (mgQE.gDW^{-1})	AN (mgME.gDW^{-1})	DPPH
1	32(-1)	10(-1)	300(+1)	131.5	7.02	0.76	75
2	32(-1)	10(-1)	100(-1)	128.3	8.29	0.67	75
3	42(0)	58(+1,414)	200(0)	104.5	7.32	0.12	75
4	52(+1)	10(-1)	100(-1)	244.1	17.49	0.46	81
5	42(0)	30(0)	341.4(+1,414)	133.8	8.04	0.27	81
6	42(0)	30(0)	58.6(-1,414)	112.6	6.36	0.21	78
7	27.86(-1,414)	30(0)	200(0)	96.9	5.64	0.13	71
8	42(0)	2(-1,414)	200(0)	283.9	18.92	0.71	82
9	32(-1)	50(+1)	300(+1)	113.5	5.17	0.15	73
10	56.14(+1,414)	30(0)	200(0)	129.4	8.85	0.11	81
11	52(+1)	10(-1)	300(+1)	245.4	15.32	0.57	82
12	52(+1)	50(+1)	100(-1)	114.8	5.60	0.13	76
13	52(+1)	50(+1)	300(+1)	118.0	6.62	0.18	76
14	32(-1)	50(+1)	100(-1)	105.7	5.45	0.14	73
15	42(0)	30(0)	200(0)	124.6	5.54	0.29	80
16	42(0)	30(0)	200(0)	125.4	5.72	0.27	80
17	42(0)	30(0)	200(0)	125.0	5.64	0.28	81
18	42(0)	30(0)	200(0)	125.8	5.54	0.30	80

Table 4 : Regression coefficients of coded factors, the coefficient of determination (R^2), standard deviations (SD) and lack of fit values of the optimized second order polynomial models of total phenolics level , flavonoids level , anthocyanins level and antiradical activity for aqueous extracts of InulaViscosa.

composite orthogonal design	Predicted value TP			Predicted value FLA			Predicted value AN			Predicted value DPPH		
	Coefficient	P value	F value	Coefficient	P value	F value	Coefficient	P value	F value	Coefficient	P value	F value
Intercept β_0	125.8			8.83			0.01			81		
Linear												
Temperature β_1	24.1	0;0023 a	19.30	3.83	0.0206 a	13.79	-0.03	0.0409 a	8.86	2.72	0.0003 a	133.72
Solid to liquid ratio β_2	-45.9	0.0000 a	70.04	-7.05	0.0024 a	46.63	-0.22	0.0000 a	385.83	-1.99	0.0011 a	71.31
Stirring rate β_3	3.8	0.5092 b	0.48	0.23	0.8314 b	0.005	0.03	0.0658 b	6.32	0.28	0.2942 b	1.46
Quadratic												
Temperature x Temperature β_1x β_1	-6;8	0.3370 b	1.04	-1.58	0.5088 b	0.53	0.05	0.0859 b	5.14	-2.75	0.0053 a	30.21
Solid to liquid ratio x Solid to liquid ratio β_2x β_2	33.6	0.0010 a	25.10	4.28	0.1220 b	3.83	0.20	0.0011 a	69.74	-1.25	0.0669 b	6.24
Stirring rate x Stirring rate β_3x β_3	-1.8	0.7914 b	0.07	-2.46	0.3230 b	1.27	0.11	0.0090 a	22.49	-0.75	0.2082 b	2.25
Interaction												
Temperature x Solid to liquid ratio β_1x β_2	-27	0.0038 a	16.16	-3.82	0.0392 a	9.11	0.05	0.0199 a	14.06	-1.00	0.0258 a	11.98
Temperature x Stirring rate β_1x β_3	0.25	0.9067 b	0.01	0.05	0.8486 b	0.04	0.01	0.6206 b	0.29	0.00	1.0000 b	0.00
Solid to liquid ratio x stirring rate β_2x β_3	1.20	0.9067 b	0.01	0.52	0.3956 b	0.90	-0.02	0.2794 b	1.56	-0.25	0.4356 b	0.75
Coefficient of determination R^2	0.94			0.96			0.99			0.98		
Residual SD as % mean	1.90			0.89			0.039			0.817		
P value of lack of fit test	0.018			0.64			0.677			0.059		

a: significant parameter and b: non-significant parameter

The negative influence of the solid to liquid ratio on the extraction yield of different polyphenol groups and on the antiradical power is important (For a temperature of 52°C and a stirring speed of 300 rpm, the shift of solid to liquid ratio from 50 to 10 $g.L^{-1}$, causes a yield increase from 118 mg GAE. g DW^{-1} to 245 mg GAE. g DW^{-1}, whereas the antiradical activity value is 8% higher).This reflects the fact that if the amount of water is not sufficient, the plant material may undergo an overheating, resulting in a decreased yield of extraction[39].

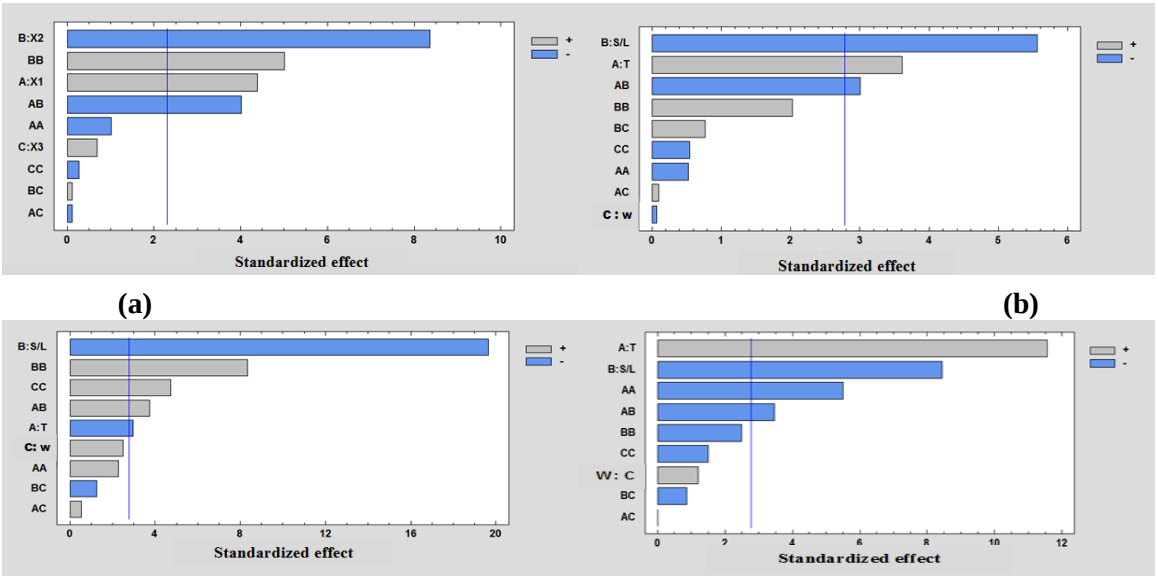

Fig. 1. Pareto chart for the effects of temperature (X1) ,solid to liquid ratio (X2) , stirring speed (X3) and their interaction effects on the level of polyphenols (a) , flavonoids content (b), yield of anthocyanins (c) and antiradical activity as mesuread by DPPH(d)of Inula Viscosa aquous extracts.

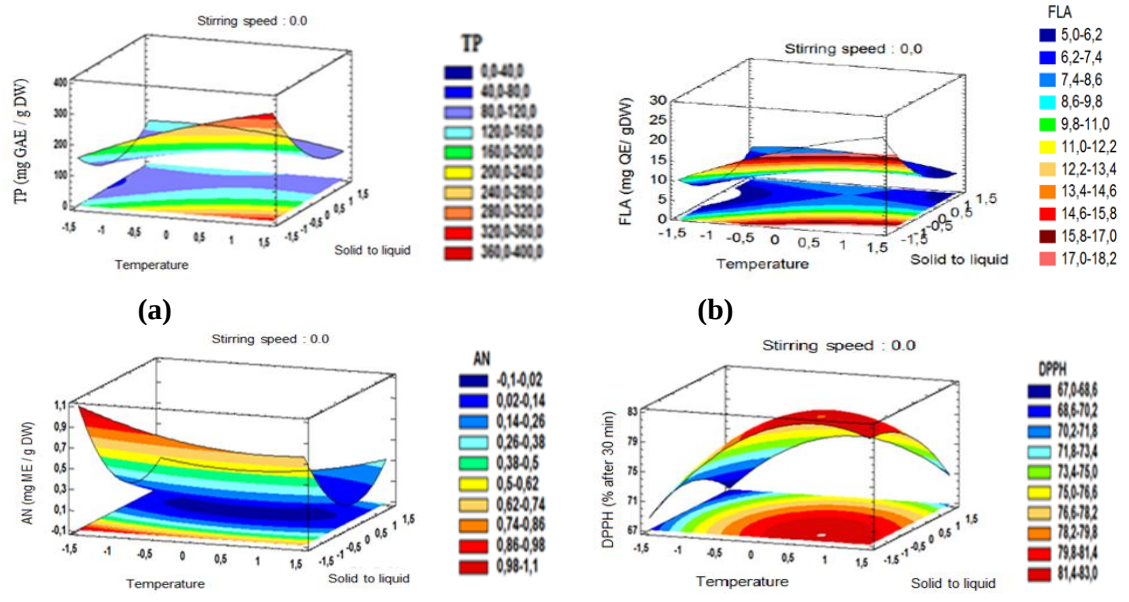

Fig. 2. Response surfaces plot showing the interaction effect of temperature and solid to liquid on the level of polyphenols (a) , flavonoids content (b), yield of anthocyanins (c) and antiradical activity as mesuread by DPPH(d)of Inula viscosa aquous extracts. The variables are presented in their coded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

Further, increasing the solid to liquid ratio leads to an insufficient amount of solvent to dissolve the existing soluble material. Indeed for an important solid to liquid ratio , extraction kinetics is accelerated because a higher concentration gradient exists between the phenolic compounds in exogenous and endogenous secretary sites[29].These findings are similar to previously published studies on other extracted natural materials[23,40].Nevertheless, several authors have shown that there is a limit to the reduction in the solid to liquid ratio below which the yield remains unchanged or decreases. In our case, this phenomenon is observed only at low temperatures (For a temperature of 32 ° C and a stirring speed of 250 rpm , from the critical value of 36 g $.L^{-1}$, the yield of polyphenols increases by increasing the solid to liquid ratio). (Desai et al , 2013) provide that for high water content, the heat could be wasted in heating up the water which may reduce the efficiency of the process. Also, hydrolysis reactions might have contributed to lower yield. Similar results were found by [28].

Table 5 : Antioxidants characteristics of Inulaviscosa aqueous extracts

Order of experiences	Process variables real and coded values			Antioxidant potential				
	X1 (°C(-))	X2 (g.L^{-1}(-))	X3 (rpm(-))	I (%)	IC50 (µg.mL^{-1})	EC50 (mg AO.(g $DDPH^{\circ}$)$^{-1}$)	TEC50 (min)	AE (gDPPH°.(mg AO. min)$^{-1}$) X 10^3
1	32(-1)	10(-1)	300(+1)	75	16	480	32	0.065
2	32(-1)	10(-1)	100(-1)	75	14.5	420	30	0.079
3	42(0)	58(+1.414)	200(0)	75	15.5	460	30	0.072
4	52(+1)	10(-1)	100(-1)	81	13	350	28	0.102
5	42(0)	30(0)	341.4(+1.414)	81	10	180	10	0.556
6	42(0)	30(0)	58.6(-1.414)	78	11	330	28	0.108
7	27 .86 (-1.414)	30(0)	200(0)	71	16	480	35	0.060
8	42(0)	2(-1.414)	200(0)	82	11.5	315	25	0.127
9	32(-1)	50 (+1)	300(+1)	73	15.5	450	30	0.074
10	56 .14 (+1.414)	30(0)	200(0)	81	11	325	27	0.114
11	52(+1)	10(-1)	300(+1)	82	12	330	28	0.108
12	52(+1)	50 (+1)	100(-1)	76	13	333	29	0.104
13	52(+1)	50 (+1)	300(+1)	76	15	350	20	0.143
14	32(-1)	50(+1)	100(-1)	73	14	460	33	0.066
15	42(0)	30(0)	200(0)	80	10.5	250	12	0.333
16	42(0)	30 (0)	200(0)	80	10.5	250	12	0.333
17	42(0)	30 (0)	20050)	81	10.5	250	12	0.333
18	42(0)	30 (0)	200(0)	80	10.5	250	12	0.333
Standards	Gallic acid (GA)			92	3	60	5	3.333
	Ascorbic acid (ASA)			91	8	100	8	1.250

3.3 Determination of the optimum extraction conditions

Figure 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 (Fig.3) correspond to a temperature of 50°C , a solid to liquid ratio of 3 $g.L^{-1}$ and a stirring speed of 217 rpm. Under these extraction conditions, the intended optimum has a content of phenolic compounds of 291 mg GAE.g DW^{-1} and an antiradical activity of 83% close to the average values found by the experimental results (TP : 289±3 mg GAE. g DW^{-1} , I%= 83±1 %).

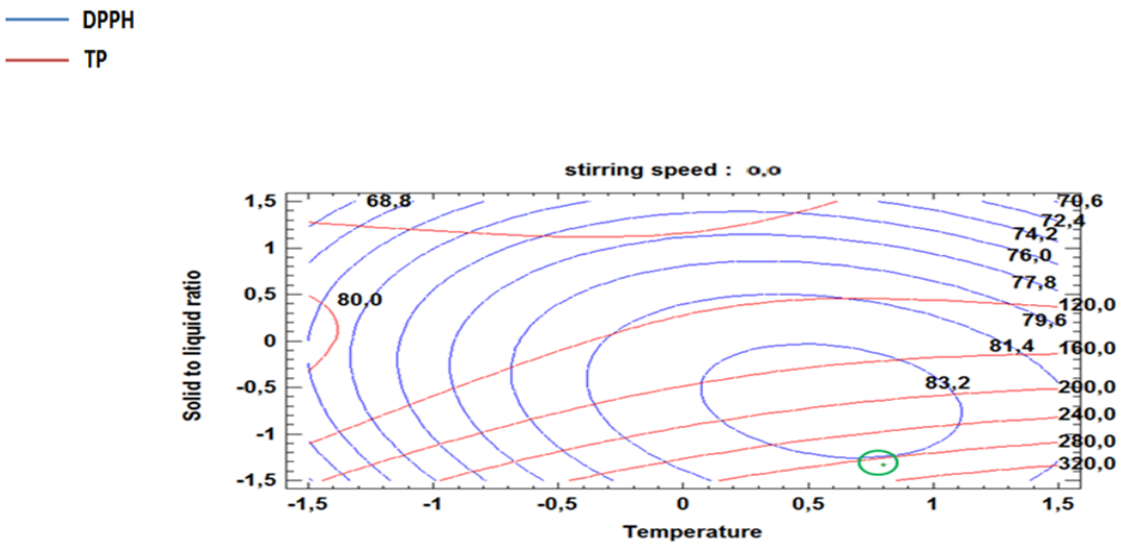

Fig 3. Superposition of contour charts for total polyphenols content (TP) and antiradical activity (DPPH) showing the optimal zone (green circle) for the two considered factors.

3.4 Purification of the extract obtained under optimum extraction conditions:

To remove different types of contaminants and interfering substances contained in the extract obtained under the determined optimal conditions, liquid phase purification was performed. The aqueous phase was washed with an equal volume of ethyl acetate three times. 92% of phenolic compounds were thus recovered. A Vacuum distillation using the Büchi R-210 rotary evaporator (BÜCHI Labortechnik AG) allowed to recover the purified extract. An evaluation of the total concentration of polyphenols and of antiradical activity as well as the determination of the antioxidant characteristics of the extract was made. The polyphenols yield is 268 mg ± 4 GAE.gDW^{-1}. The purification resulted in a loss of 7.9% of phenolic compounds but with improved antiradical power of 8.4% (I=90%). The antioxidant characteristics of the purified extract are: (IC50= 5µg.mL^{-1}, EC50 = 125 mg AO. g DW^{-1} and TEC50 = 8min).

4. Conclusion

Solid–liquid extraction using water as a solvent instead of organic solvents proved to be an efficient method for the recovery of antioxidant polyphenols from leaves of Algerian Inulaviscosa (L.). Response Surface Methodology was applied to investigate the effect of operating parameters on the studied responses (total phenolics, flavonoids, anthocyanins and antiradical activity) and models describing these responses were obtained with high R^2 values(> 0.9). In general, the linear effect of temperature, the solid to liquid ratio and the interaction effects between both parameters proved that they are the most significant factors. The maximum yield of phenolic compounds extracted from Inulaviscosa (L.) corresponds to 284 mg GAE.g DW^{-1}. The multiple responses optimization of the extraction conditions (T=50°C, S/L= 3g.L^{-1} and ω = 217 rpm) was of crucial importance for obtaining an extract rich in phenolic compounds (291 mg GAE. g DW^{-1}) with high antiradical activity (83%). Purified extract obtained under these conditions (Purification yield correspond to 92%), contain simultaneously substantial amounts of phenolic compounds (268 mg GAE. g DW^{-1}) with a high antiradical power (90%). The antioxidant characteristics of the extract (IC50= 5µg.mL^{-1}, EC50 = 125 mg AO. g DW^{-1} and TEC50 = 8min) were comparable to the properties of powerful antioxidants (Gallic acid and ascorbic acid). With an antiradical efficiency of 10^{-3} g DPPH°.(mg AO.mn)$^{-1}$, the purified extract belongs to the group of antioxidants with medium antiradical activity. However despite the use of ethyl acetate has improved the antioxidant potential of the final extract, the substitution of this method of purification by a clean alternative method like solid phase purification or the use of membrane separation methods based on osmosis or ultrafiltration is preferred , especially whenever the extracted component is to be used in food and pharmaceutical applications.

Water, this unique green solvent with low cost has proved to be effective for the extraction of polyphenols from Inulaviscosa's leaves. These phenolic compounds recovered using an environmentally-friendly cleaning process constitute a good source of natural antioxidants for food, cosmetic and pharmaceutical industries.

Acknowledgements

The authors gratefully acknowledge the Chemical Engineering Department (National Polytechnic School of Algiers) for their funding of this project, MrKamelMezieche for providing the Inulaviscosa samples and Pr Ahmed Zaid for language revision.

References

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

[2] L. Boulus, Medicinal plants of North Africa, Reference Publications, Michigan, USA, 1983.

[3] S. Manez, V. Hernandez, R.M. Giner, J.L. Rios, C. RecioMdel, Inhibition of pro-inflammatory enzymes by inuviscolide, a sesquiterpene lactone from Inulaviscosa, Fitoterapia. 78 (2007) 329-331.

[4] N.A. Zeggwagh, M.L. Ouahidi, A. Lemhadri, M. Eddouks, Study of hypoglycaemic and hypolipidemic effects of Inulaviscosa L. aqueous extract in normal and diabetic rats, J Ethnopharmacol. 108 (2006) 223-227.

[5] Chahmi N, Anissi J, Jennan S, Farah A, Sendide K, Hassouni ME. Antioxidant activities and total phenol content of Inulaviscosa extracts selected from three regions of Morocco, Asian Pac J Trop Biomed. 2015; 5(3) : 228-233.

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

[7] E. Wollenweber, K. Mayer and J.N. Roitman, Exudate flavonoids of Inulaviscosa, Phytochem. (1991) 2445–2446.

[8] A. Andolfi, N. Zermane, A. Cimmino, F. Avolio, A. Boari, M. Vurro, A. Evidente, Inuloxins A-D, phytotoxic bi- and tri-cyclic sesquiterpene lactones produced by Inulaviscosa: potential for broomrapes and field dodder management, Phytochemistry. 86 (2013) 112-120.
[9] I.E. Haoui, R. Derriche, L. Madani, Z. Oukali, Analysis of the chemical composition of essential oil from Algerian Inulaviscosa (L.) Aiton, Arabian J. Chem. 8 (2011) 587-590.
[10] J.F. Sanz, C. Ferrand , J. Alberto Marco , Oxygenated nerolidol esters and eudesmane acids from Inulaviscosa, Phytochemistry. 30 (1991) 3653-3655.
[11] M. Grande, P. Torres, F. Piera , I.S. Bellido, triterpenoids from dittrichiaviscosa, Phytochemistry. 31 (1992) 1826-1828.
[12] A. Valdés, G. Sullini, E. Ibáñez, A. Cifuentes, V. García-Cañas, Rosemary polyphenols induce unfolded protein response and changes in cholesterol metabolism in colon cancer cells, J Funct Foods. 15 (2015) 429-439.
[13] J. Pi, J. Zeng, J.J. Luo, P.H. Yang, J.Y. Cai, Synthesis and biological evaluation of Germanium(IV)-polyphenol complexes as potential anti-cancer agents, Bioorg Med Chem Lett . 23 (2013) 2902-2908.
[14] E.S. Kao, C.J. Wang, W.L. Lin, C.Y. Chu, T.H. Tseng, Effects of polyphenols derived from fruit of Crataeguspinnatifida on cell transformation, dermal edema and skin tumor formation by phorbol ester application, Food ChemToxicol. 45 (2007) 1795-1804.
[15] A. Ebrahimi, H. Schluesener, Natural polyphenols against neurodegenerative disorders: potentials and pitfalls, Ageing Res Rev. 11 (2012) 329-345.
[16] G. Indrayanto, I. Kusumawati, Natural Antioxidants in Cosmetics, in: Studies in Natural Products Chemistry, Atta-ur-Rahman. , 2013, pp. 485-505.
[17] C. Capello, U. Fischer, K. Hungerbühler, What is a green solvent?A comprehensive framework for the environmental assessment of solvents, Green Chem. 9 (2007) 927.
[18] 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. 102(2007) 771–776.
[19] A. Djeridane, M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker , N. Vidal, Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. , Food Chemistry. 97(2006) 654-660.
[20] R. Di Stefano, M.C., Cravero, N. Genfilini, Metodi per lo studio deipolifenolideivini, L'Enotecnico. 5 (1989) 83-89.
[21] F.Que ,L.Mao, X.Pan, Antioxidant activities of five Chinese rice wines and the involvement of phenolic compounds, Food Res Int. 1(2006) 581-587.
[22] R. Scherer , H.T. Godoy, Antioxidant activity index (AAI) by the 2,2 diphenyl-1-picrylhydrazyl method, Food chem. 112(2009) 654-658.
[23] C. Bonnaillie, M.Salacs, E. Vassiliova, I. Saykova, Etude de l'extraction de composés phénoliques à partir de pellicules d'arachide (Arachishypogaea L.), Rev Génie Ind.7 (2012) 35-45.
[24] S.F Reis , D.K. Rai, N. Abu-Ghannam , Water at room temperature as a solvent for the extraction of apple pomace phenolic compounds, Food Chem (2012) 1991-1998.
[25] F. Gironi, V. Piemonte, Temperature and solvent effects on polyphenol extraction process from chestnut tree wood.7 (2011) 857-862.
[26] L.F. Abu-Qatouseh , H. Boutennoune, L. Boussouf, K. Madani, P. Shihab, K. Al-Qaoud, In vitro susceptibility of Helicobacter pylori to urease inhibitory effects of polyphenolic extracts of local herbs from Algeria, Int Arab J Antimicrob Agents. (2013).

[27] M. Latoui, B. Aliakbarian, A.A. Casazza, M. Seffen, A. Converti, P. Perego, Extraction of phenolic compounds from Vitexagnus-castus L., Food Bioprod Process. 90 (2012) 748–754.
[28] G. Angelov, S.S. Boyadzhieva, S.S. Georgieva, Rosehip extraction: Process optimization and antioxidant capacity of extracts, Cent. Eur. J. Chem. 12 (2014) 502-508.
[29] D.D. Gertenbach, Solid–liquid extraction technologies for manufacturing nutraceuticals from botanicals, in: J. Shi, Mazza, G., Maguer, M.L. (Eds) , Functional Foods Biochemical and Processing Aspects, CRC press, Boca Raton, 2001, pp. 333-366.
[30] V. Sant'Anna , A. Brandelli, L.D.F. Marczak , I.C. Tessaro, Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts, Sep Purif Technol.100(2012) 82-87.
[31] Q.V. Vuonga, S.Hiruna, P.D. Roacha, M. C. Bowyera, P.A. Phillips, C.J. Scarlett , Effect of extraction conditions on total phenolic compounds and antioxidant activities of Carica papaya leaf aqueous extracts, J Herb Med. 3 (2013) 104-111.
[32] P. Penchev, G. Angelov, J.S. Condoret, Extraction des agents antioxydants (acide rosmarinique) à partir de la mélisse (Melissa officinalis L.), Rev Génie Ind.5 (2010) 115-123.
[33] D.R. Pompeu, E.M. Silva, H. Rogez, Optimisation of the solvent extraction of phenolic antioxidants from fruits of Euterpe oleracea using Response Surface Methodology, Bioresour Technol. 100 (2009) 6076–6082.
[34] M.T.C. Machado , B.C.B.S Mello, M.D. Hubinger, Study of alcoholic and aqueous extraction of pequi (CaryocarbrasilienseCamb.) natural antioxidants and extracts concentration by nanofiltration, J Food Eng . 119 (2013) 450-457.
[35] M.Dent, V. Dragovi-Uzelac, M. Peni, M. B.T. Bosiljkov, B. Levaj, The Effect of Extraction Solvents, Temperature and Time on the Composition and Mass Fraction of Polyphenols in Dalmatian Wild Sage (Salvia officinalis L.) Extracts, Food Technol. Biotechnol. 51 (2013) 84-91.
[36] N.E. Durling, O.J. Catchpole, J.B. Grey, R.F. Webby, K.A. Mitchell, L.Y. Foo , N.B. Perry , Extraction of phenolics and essential oil from dried sage (Salvia officinalis) using ethanol-water mixtures, Food Chem. 101(2007) 1417-1425.
[37] W. Ebling, A. Jamet, Stabilization mechanisms for anthocyanin: The case for copolymerization reactions, in: J. Welti-Chanes, G.V. Barbosa-Cánovas, J.M. Aguilera (Eds.) , Engineering and food for the 21st century, CRC Press, Florida , 2002, pp. 141-150.
[38] G.H. Laleh, H. Frydoonfar, R. Heidary, R. Jameei , S. Zare, The effect of light, temperature, pH and species on stability of anthocyanin pigments in four Berberis species., Pak. J. Nutr. 5 (2006) 90-92.
[39] M.A. Desai, J.Parikh , A. Kumar De, Modelling and optimization studies on extraction of lemongrass oil from Cymbopogonflexuosus (Steud.) Wats, Chem Eng Res Des. 92 (2014), 793-803.
[40] S. I. Mussatto , L.F. Ballestros, S.Martins, J.A. Teixeira, Extraction of antioxidant phenolic compounds from spent coffee grounds, Sep Purif Technol. 83(2011) 173-179