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OPTIMIZATION OF THE EXTRACTION OF FLAVONOIDS FROM ARTEMISIA HERBA ALBA FROM TWO STEPIQUES REGIONS (M'SILA AND DJELFA)

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Abstract

Description of the subject: In the context of the valorization of steppe plants of medicinal interest, this work deals with the influence of soil and climatic factors on the production of flavonoids in *Artemisia herba alba.*
Objectives: The objective of this study is to evaluate the effect of ecological parameters on the production of flavonoids from *Artemisia herba-alba* L.
Methods: The extraction process (solid/liquid) is carried out with three solvents (water, methanol and ethanol) and followed by analyses (UV Visible spectrophotometry and high-performance liquid chromatography).
Results: The results indicate the richness of Artemisia herba alba in phenolic compounds. Analysis of the liquid chromatography of the three extracts shows that the plant studied contains the following polyphenols: catechic acid, rutin, caffeic acid and quercetin.
Note that by UV-visible spectrophotometer was measured the impregnation rate of solvents where methanol gave the best extraction yield in plants native to M'sila with (0.86%). Also by UV-visible spectrophotometer was measured the rate of flavonoids and it is the chloroform extract of this same sample gave the highest in (1,966µg eqQu / mg PS) compared to the extracts of organic and aqueous ethyl acetate of the two regions "M'sila and Djelfa".
Conclusion: This study reveals the richness of *Artemisia herba alba* in flavonoids; as well as the specificity of each region.
Keywords: *Artemisia herba-alba*, extraction, flavonoids, yield, steppe regions.

OPTIMISATION DE L'EXTRACTION DES FLAVONOÏDES D'ARTEMISIA HERBA ALBA DE DEUX RÉGIONS STEPIQUES (M'SILA ET DJELFA)

Résumé :

Description du sujet : Dans le cadre de la valorisation des plantes steppiques a intérêt médicinal, ce présent travail traite l'influence des facteurs pédologiques et climatiques sur la production de flavonoïdes chez *Artemisia herba alba*.
Objectifs : Cette étude vise l'évaluation de l'effet des paramètres écologiques sur la production des flavonoïdes d'*Artemisia herba-alba* L.
Méthodes : Le processus d'extraction (solide/liquide) est effectué avec trois solvants (eau, méthanol et éthanol) et suivi par des analyses (spectrophotométrie UV Visible et par chromatographie liquide à haute performance).
Résultats : Les résultats indiquent la richesse d'*Artemisia herba alba* en composés phénoliques. L'analyse de la chromatographie liquide des trois extraits montre que la plante étudiée contient les polyphénols suivants: l'acide catechique, la rutine, l'acide caféique ainsi que la quercetine.
Notons que par spectrophotomètre UV-visible a été dosé le taux d'imprégnation des solvants où le méthanol a donné le meilleur rendement d'extraction chez les plantes originaires de M'sila avec (0.86%). Aussi par spectrophotomètre UV-visible a été dosé le taux des flavonoïdes et c'est l'extrait Chloroformique de ce même échantillon a donné le plus élevé en (1.966µg éqQu/mg PS) par rapport aux extraits d'acétate d'éthyle organique et aqueux des deux régions "M'sila et Djelfa".
Conclusion : Cette étude révèle la richesse d'*Artemisia herba alba* en flavonoïdes; ainsi que la spécificité climatique de chaque région.
Mots clés : *Artemisia herba-alba*, extraction, flavonoïdes, rendement, régions steppiques.

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INTRODUCTION

Organic agriculture and herbal medicine have sparked renewed interest in steppe plants. Among these plants: *Artemisia herba-alba* commonly called "Chih" in Arabic and "the white armoise" in French belonging to the Asteraceae family is a shrub foraging the Mediterranean region with a morphology characterized by its thymol-like flowers, its axes and its very dense root system [1]. This plant is able to exploit soil moisture up to 50 cm deep [2] and can take advantage of crust fractures, to reach pockets of moisture, especially in soils with calcareous encrustation [3]. This provided it a specific genetic variability [4]. In Algeria, *Artemisia herba alba* is described in semi-arid and arid bioclimatic environment. It is found in steppe soils south-west of Sebou of the Oranie [5], to the eastern Algerian of Batna [6], passing through the region of Djelfa [7, 8, 9]. It is present in the spontaneous state in large quantities in M'sila [10], and Boussaâda [11] .The southern Algerian is also marked by the presence of the white mugwort in its territory in Biskra [12] and Ain Es-Sefra [13]. The phytotherapic characteristics of *Artemisia herba alba* are due to the synthesis of secondary metabolites, such as the flavonoids present in the leaf cuticle and in the epidermal cells of the leaves. This metabolic process is related to very conditions of life plant, facing and even to resist to the different abiotic and biotic stresses [15]. From the application point of view, several flavonoids isolated from plants have antioxidant activities and the ability to prevent the toxic effects of oxidative stress in diabetes [6]. *Artemisia herba alba L* showed antioxidant activity that could be considered a good source of free radical scavengers [16]. Due to the spontaneity of *Artemisia herba alba L* and the effect of the environment on its development, growth and phytochemical composition, it is important to evaluate the effect of ecological parameters on the production of flavonoids. This study consists in the establishment of an optimized extraction protocol of the flavonoids of *Artemisia herba-alba* by various solvents, with a qualitative and quantitative analysis (HPLC) in order to select the most efficient extraction by solvent. To study the impact of the region on the production of flavonoids; comparative study of the flavonoids production of *Artemisia herba-alba* was done from two regions of the Algerian steppe: M'Sila (35 ° 42 '20 North, 4 ° 32' 30 East) with a warm mediterranean climate and Djelfa (34 ° 40 '22 North, 3 ° 15' 46 East) with a semi-arid climate.

MATERIAL AND METHODS

1. Plant material

The plants of *Artemisia herba alba* were harvested in March at the stage of low-level flowering in the area of Sed K'sab (province of Msila) at the daily temperature of 24 ± 2°C and used to optimization of the protocol of extraction. For the comparative study, the uses of a second sample was necessary of *Artemisia herba alba* harvested in March (beginning of flowering) in Oued Ben Nâama (province of Djelfa) at the daily temperature of 19±2°C. The species was identified at the Plant Biotechnology Department. The aerial part of the plant, washed thoroughly to remove all traces of soil, is dried in the shade at 20 ° C ± 5 ° C for 20 days and then finely powdered in Kraft paper bags

2. Preparation of phenolic extracts

Extraction tests of phenolic compounds were carried out with different solutions: methanolic, ethanolic and water. The choice of the solvent was conditioned by the polar nature of the phenolic compounds.

To extract the polyphenols from the aerial part of *Artemisia herba alba* we opted for the protocol described by Romani et *al.* [17] with some modifications: The *Artemisia herba alba* powder macerated at room temperature for 24 h with different solvents: methanol (99.5%), ethanol (96%) and sterile distilled water. After filtration on a filter paper No. 1, the filtrates are evaporated under reduced pressure using a rotary evaporator. They stored at 4°C until they are used.

2.1. Volume of impregnation (V_{imp})

The residues obtained were weighed and dried at 105°C for 2 h. The impregnation volume (V_{imp}) was defined as the volume of solvent absorbed by a given mass of the solid (mL solvent / g dry matter (DM).

Clémentine Bonnaillie [18] determines the formula by the relation:

$$V_{imp} = \frac{M_{imp} - M_{dm}}{V_{solvent}}$$

V_{imp}: Impregnation volume, M_{imp}: Impregnation mass, M_{dm}: Mass in dry matter, $V_{solvent}$: Volume of the solvent

2.2. Chromatographic analysis

The liquid phase of each extract was recovered for qualitative analysis by HPLC (High Performance Liquid Chromatography / Pressure).

The analysis of the extracts is done with the C18 column and a mobile phase composed of acidified water (pH 2.3) / acetonitrile (65/35, v / v) and reading results at 254 nm [19]. A standard range was established separately with quercetin, catechic acid, rutin, caffeic acid. The chromatographic interpretation is according to the number of peaks and secondary metabolites obtained.

3. Comparative study

The plants of *Artemisia herba alba* L from two regions M'sila and Djelfa were compared between yields and flavonoid contents. All experiments were carried out in triplicate. The experimental results were expressed as means ± standard deviation (n = 3), and the means were compared using Student's t test. *p*-Values < 0.01 are considered significant. All statistical analyses were performed using the free online statistical software statistica version 10.

3.1. Extraction efficiency

The extraction yield was calculated by the formula of Console et *al*. [20]:

$$\text{Yield (R \%)} = \frac{\text{Mass of residue}}{\text{Mass of vegetable powder}} * 100$$

3.2. Extraction of flavonoids

To extract flavonoids from *Artemisia herba alba*, we adopted the protocol described by Mabray and Makhram [21] with some modifications (Fig. 1). The finely ground plants are macerated in methanol, followed by a series of liquid / liquid extraction with n-hexane (3 times v/v) for the delipidation, followed by ethyl acetate (10 times v/v). The aqueous phase was recovered and the organic phase was added to chloroform (3 times v/v) to obtain two phases: one organic and the other aqueous. The extracts obtained were named according to the separation solvent: chloroformic extract (flavonoids aglycones), organic ethyl acetate extract (mono and diglycosidic flavonoids), aqueous ethyl acetate extract (di-tri and tetraglycosidic flavonoids). The various extracts were recovered in glass bottles and then stored at 4°C until they are used.

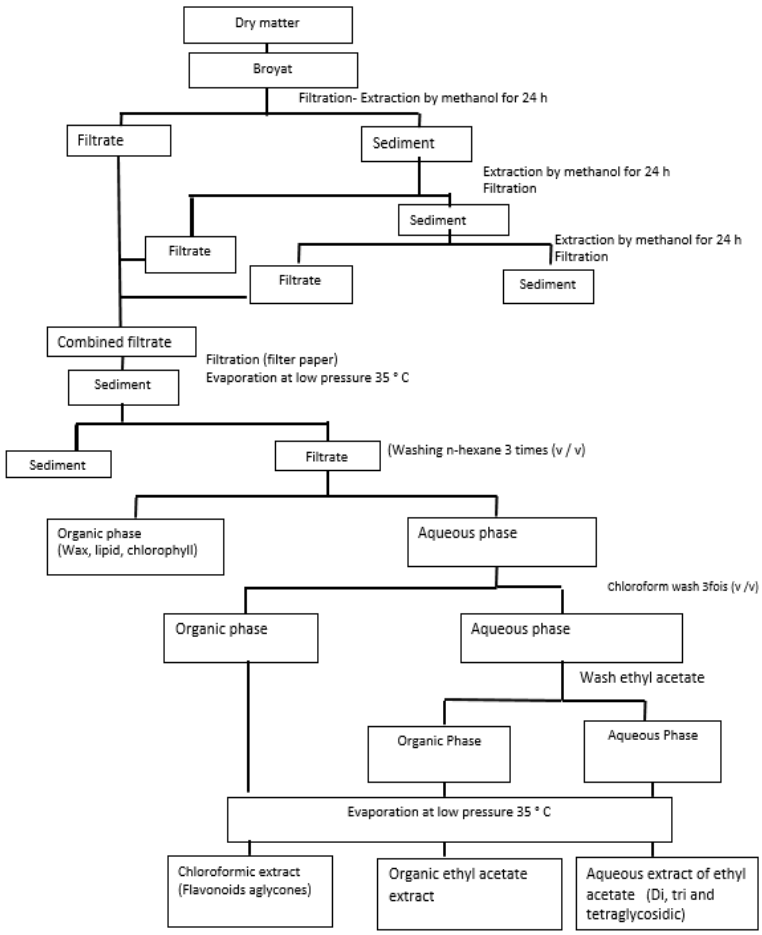

Figure. 1: Flavonoids extraction protocol according and edited to Mabray and Makhram, [21]

3.3. Determination of flavonoids

The total flavonoid assay was carried out according to the method described by Dehpeur et *al*. [22]: 500 µL of each extract to be analyzed are added to 1500 µL of methanol (95%), 100 µL of aluminum trichloride ($AlCl_3$) (10%) (m / mL). v), 100 µl of 1M sodium acetate and 2.8 mL of distilled water. The mixture was stirred and then incubated in the dark at 20±5°C for 30 minutes. The control was carried out by replacing the extract with methanol (95%) and the absorbance is measured at 415 nm (Perkin Elmer UV spectrophotometer). A standard range was established separately with quercetin (0.56 to 11.3 µg / mL) to calculate the concentration of flavonoids in each extract.

The results of the assay was expressed in microgram equivalents of quercetin per milligram of extract.

RESULTS

1. Volume of impregnation

The great power of the solvent to penetrate within the plant matrix is attributed to the solubility of polyphenols and the degree of solute polymerization due to the increase in the number of OH-hydroxyl groups [23]. These tests also made it possible to calculate the volume of impregnation ($V._{imp}$). The impregnation volume varies between 0.169 mL / g for methanol, 0.121 mL / g for ethanol and 0.032 mL / g for water (Fig. 2).

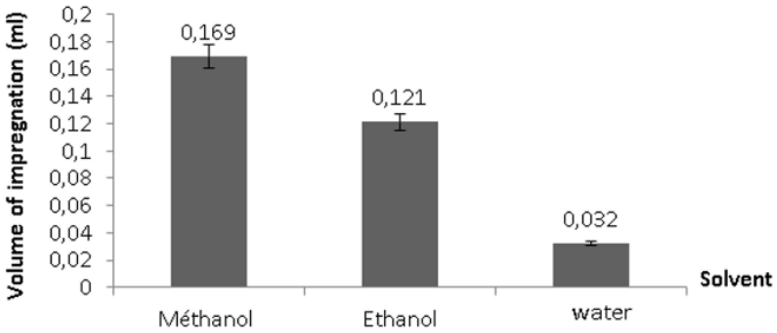

Figure 2: Volume of impregnation. (Student's t test, $p < 0.01$)

2. Chromatographic analysis

The chromatogram of the three extracts shows that *Artemisia herba alba* contains polyphenols including catechic acid, rutin, caffeic acid and quercetin. The chromatograms have shown that the great power is due to the methanol which we obtained ten different secondary metabolites exprimed by ten peaks (Fig. 3a) followed by water with 8 peaks (Fig. 3c) and ethanol with 7 peaks (Fig. 3b). We also found the presence of quercetin in methanolic extract. Therefore, the methanol appears as the best extraction solute.

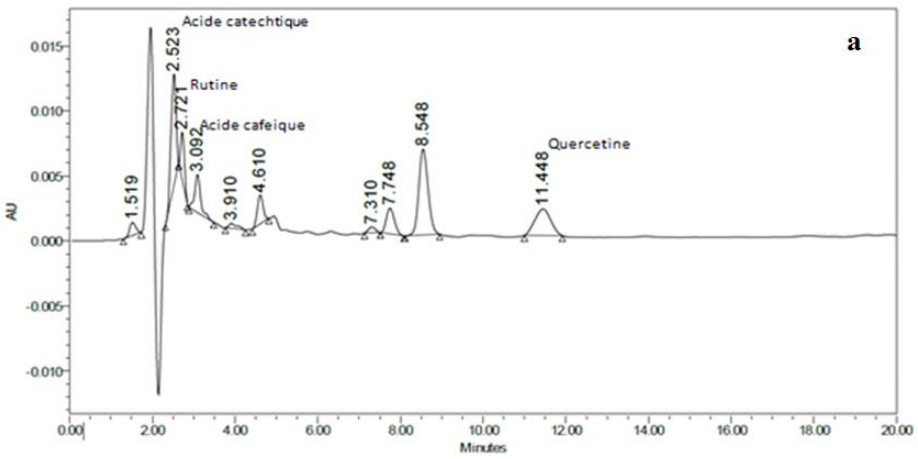

Figure. 3: Chromatogram of the liquid solution of Artemisia herba alba in a:methanol

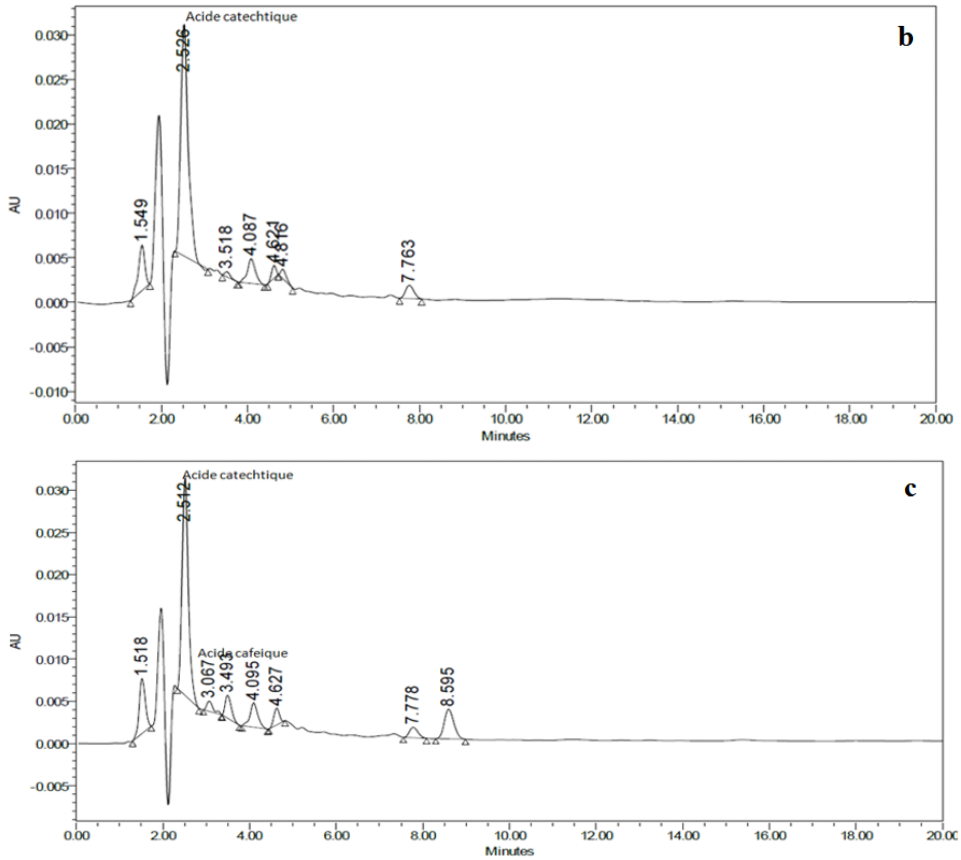

Figure. 3-suite: Chromatogram of the liquid solution of Artemisia herba alba in b: ethanol, c: water

3. Extraction yield in relation to dry matter

In terms of the extraction yield of the various extracts with respect to the dry matter, plants from M'sila give the best yield with 0.86 % compared to Djelfa plants with 0.66 % (Student's t test, $p < 0.01$) (Fig.4).

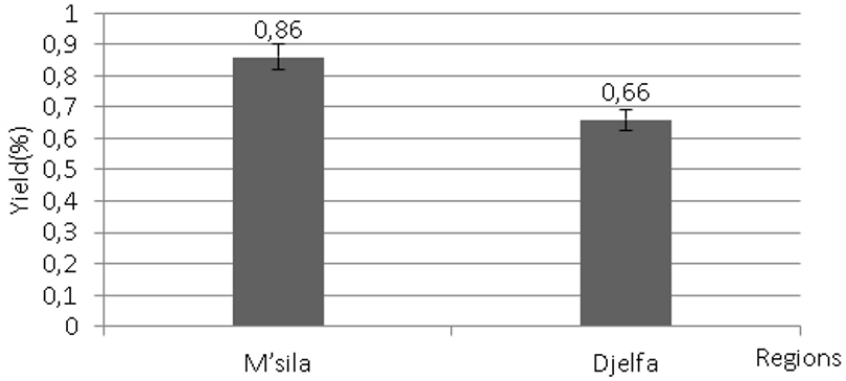

Figure 4. Extraction yield of M'sila and Djelfa samples relative to the dry matter

4. Extraction yield of the different extracts

The extraction yield of all the extracts shows that the sample of M'sila contains the yield of the different methanolic extracts highest secondary metabolites. These same results show that the yield of the ethyl acetate extracts obtained is higher compared to the other extracts with 20.35% for the M'sila sample, followed by 20% for the Djelfa sample (Student's t test, p <0.01). The chloroformic extract of the Djelfa sample gives the highest yield with 18.71 % compared to that of M'sila. The aqueous extract gave a very low yield with a rate of 14 % (Student's t test, p<0.01) (Fig. 5).

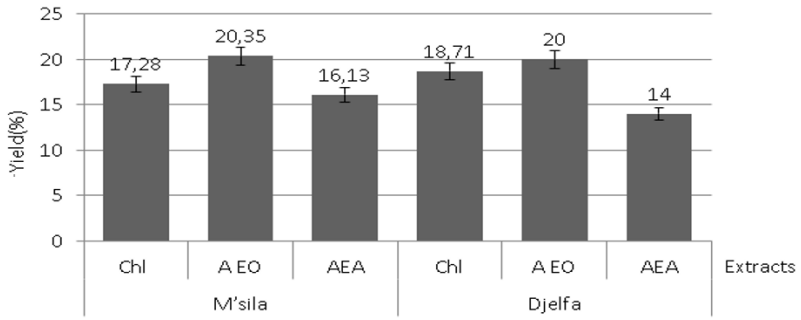

Figure 5: Extraction yields of the different extracts of the M'sila and Djelfa sample in methanol
F: Flavonoid, Chl: Chloroformic, AEO: Organic ethyl acetate, AEA: Aqueous ethyl acetate.

5. Flavonoids content

The results of the different samples shows the richness of *Artemisia herba alba* in flavonoids (Fig. 6). In fact, the chloroformic extract of M'sila samples records the highest levels (Student's t test, $p < 0.01$), with 1.966 µg eqQu / mg DW, followed by the organic ethyl acetate extract of M'sila samples with 1.057 µg eqQu / mg DW. However, the lowest dose is that of the aqueous ethyl acetate extract of Djelfa samples where we record 0.26041µg eqQu / mg DW.

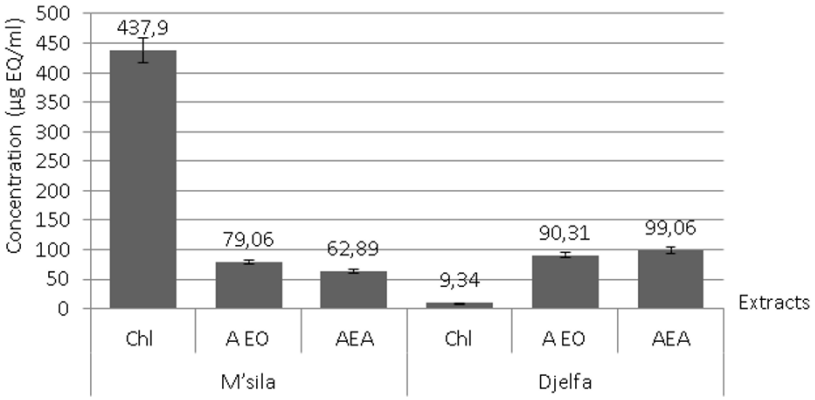

Figure 6: Dosage of flavonoids of *Artemisia herba alba*.
Chl: Chloroformic, AEO: Organic Ethyl Acetate, AEA: Aqueous Ethyl Acetate.

DISCUSSION

1. Volume of impregnation

The overall analysis of the impregnation volume of the different solvents shows that methanol with the highest volume, can be explained by its high degree of polymerization, which facilitates the solubilization of polyphenols. However maceration is a discontinuous method, the dissolve should be replaced until the plant matter is exhausted it is difficult to compare the results with those of the bibliography.

2. Chromatographic analysis

The analysis by liquid chromatography of the three extracts shows that *Artemisia herba alba* contains polyphenols including catechic acid, rutin, caffeic acid and quercetin.According to the work of abou zaid on *Artemisia herba alba*, this botanical genus contains flavonoids, flavones and flavonols in the vast majority as well as their glycosidic derivatives. Moreover the methanol appears as the best extraction solute which we obtained nine different secondary metabolites. As well as Quercitin which is an aglycone flavonoid and an excellent antioxidant

3. Extraction yield in relation to dry matter

In terms of the extraction yield of the various extracts with respect to the dry matter, plants from M'sila give the best yield compared to Djelfa plants . This is may due to the climate of M'sila which is a warm mediterranean climate and Djelfa is a semi-arid climate. According to M. Haouari and A. Ferchichi [4], the white wormwood has specific genetic variability and has proved its great variability regarding the phytological behavior. Also according to Ebrahimzadeh [24], the quantity of phenolic compounds depends essentially: on their origin, variety, growing season, climatic and environmental conditions, geographical location, different diseases that can affect the plant and maturity of the plant.

4. Extraction yield of the different extracts

The extraction yield of all the extracts shows that the sample of M'sila contains the yield of the different methanolic extracts highest secondary metabolites. This is probably due to early flowering compared to Djelfa because it is during the flowering period that there is the greatest production of flavonoids, because of their role in the pigmentation of flowers for the attraction of insects' pollinizers, as well as their protective role against the harmful insects of the plant.

5. Flavonoids content

The high content of flavonoids in the sample from M'Sila is due to the different climatic conditions of Djelfa. The recorded temperatures were higher (24°C) compared to those of Djelfa (19°C) with a precocious flowering. The increase in temperature induces the production of flavonoids by the plant because of their capacity to absorb the ultraviolet radiation (330-350 nm) and the radiation of certain parts of the visible spectrum (520-560 nm), the flavonoids protect the plant tissues of excessive radiation. This is confirmed by the localization of flavonoids in the leaf cuticle and epidermal cells of the leaves [27]. In a state of stress, tri- and tetra-glycosylated flavonoids will be degraded by hydrolysis to mono and diglycosylated flavonoids which will in turn be hydrolysed to flavonoid aglycones, so that flowering and temperature increase will increase the production of flavonoids aglycones from sample of M'sila. Flowering in the spring increases the synthesis of flavonoids in the plant including aglycones. We can deduce that there is a relationship between flowering and flavonoid production, because the increase in temperature induces flowering, which increases the synthesis of flavonoids in the plant including the aglycones. That are consumed in the state of plant stress by oxidation-reduction reactions, such as heat or as a pigment conferring different colors for plant tissues to facilitate pollination, or to be provided to animals through food. The work of Abu Zaid [25], on *Artemisia herba alba* shows that this botanical genus contains flavonoids in large majority: flavones and flavonols and their glycosidic derivatives. The results obtained with Djelfa samples are in agreement with the work of Khennouf Seddik [26], on *Artemisia herba alba* of Setif (Algeria) where they found that the phase of organic ethyl acetate is the richest in flavonoids.

CONCLUSION

The extraction of polyphenolic compounds is a crucial step for the valorization of these active ingredients; it depends on the appropriate solvent that preserves biological properties. It is clear from the study that maceration with methanol is the best technique for extracting total polyphenols and flavonoids, due to its penetration into the plant matrix and the degree of polymerization; this is confirmed by liquid chromatography with the detection of quercetin only in the methanol extract.The results of this study reveal the richness of *Artemisia herba alba* in flavonoids; as well as the influence of the Region factor on their productions. The amount of flavonoids in the M'sila sample is different from that of Djelfa, due to its phonotypical heterogeneity and adaptation to climate. Therefore, there is a close relationship between production, hydrolysis of its secondary metabolites and ecological factors (temperature, light ...). It is interesting in our future research work to better optimize the extraction and isolation protocols of flavonoids, to also target the phenological stages and to follow the evolution of flavonoids during the phenological stages of the plant according to the geographical areas. To quote the pedoclimatic characters that can confer a better synthesis of the different phenolic compounds.

REFERENCES

[1]. Le Floc'h E. (1983). *Contribution à une étude ethnobotanique de la flore tunisienne.* Ed Ministère de l'enseignement supérieur et de la recherche scientifique. Vol 1, Tunisie, 402p.

[2]. Floret Ch. & Pontannier, R. (1982). *L'aridité en Tunisie présaharienne, climat, sol, végétation et aménagement.* Trav. Docum. ORSTOM, 552p.

[3]. Ourcival J. M. (1992) *Réponse de deux chamaephytes de la Tunisie présaharienne à différentes contraintes et perturbations.* Thèse Doc. USTL, Montpellier, 167p.

[4]. Haouari M. & Ferchichi A. (2004). Utilisation des marqueurs ISSR pour l'étude du polymorphisme génétique d' *Artemisia herba-alba*. *CIHEAM*, p. 115-119.

[5]. Benabadji Bouazza. (1996). *Etude des Huiles essentielles de plantes pastorales quelques de la région de Matmata (Tunisie).* Bull. Inst .Sci., Vol. 20 : 77-86.

[6]. Bouldjadj R. (2009). *Etude de l'effet antidiabétique et antioxydant de l'extrait aqueux lyophilisé d'Artemisia herba alba asso chez des rats sains et des rats rendus diabétiques parstreptozotocine*. Thèse Magister, Université Mentouri Constantine., 167p.

[7]. Messaoudene D.; Belguendouz H.; Ahmedi M. L.; Benabdekader T.; Otmani F.; Terahi M., Youinou P. &Touil-Boukoffa C.(2011). *Ex vivo effects of flavonoïdsextracted from Artemisia herba alba on cytokines and nitric oxide production in Algerian patients with Adamantiades-Behçet's disease.* J Inflamm (Lond)., 140p.

[8]. Dahmani H. & Baaliouamer A. (2005). Chemical composition of the Algerian essential oil of *Artemisiaherba-alba* native to Dejelfa. *Riv. Ital.* EPPOS., Vol. 40 :7-13.

[9]. Salido, S.; Altarejos, J.; Nogueras, M. & Sanchez, A.(2001). *Chemical composition of essential oil of Artemisia herba-alba Asso. ssp. Valentine (Lam.) Marc. J. Essen. Oil Res.*, Vol.13: 221-224.

[10]. Dob, T& Benabdelkader, T.(2006). *Chemical composition of the essential oil of Artemisia herbaalbaAsso grown in Algeria.* J. Essen. Oil Res., Vol.18: 685-690.

[11]. Baaliouamer, A. & Dahmani- Hamzaoui, N.(2010). *Chemical composition of Algerian Artemisiaherba alba essential oils isolated by microwave and hydrodistillation*. Journal of essential reaserch.,138p.

[12]. Bezza, L.; Mannarino, A.; Fattarsi, K.; Mikail,C.; Abou, L.; Hadji-Minaglou, F. & Kaloustian, J. (2010). *Chemical composition of the essential oil of Artemisia herba-alba issued from the district of Biskra (Algeria).* Phytothérapie., 8 (5), :277-281.

[13]. Benabdellah, M.; Benkaddour, M.; Hammouti, B.; Bendahhou, M. &Aouniti, A. (2006). *Inhibition of steel corrosion in 2 M H3PO4 by Artemisia oil.* Applied Surface Science., 225, 8341-8347.

[14]. Recchia, C. & Auberval, N.(2004). Prévention du stress oxydant dans le diabète et ses complications par des antioxydants d'origine naturelle. Diabetes Care., Vol.27 : 36-362.

[15]. Bruneton J. (1993). *Pharmacognosie, phytochimie et plantes médicinales.* La Voisier TEC et DOC., p.268-277.

[16]. Djeridane, M.; Yousfi, B.; Nadjemi, D. Boutassouna, P.; Stocker. &Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenoliccompounds. *Food Chemistry*., Vol.97:654-660.

[17]. Romani A.; Pinelli P.; Cantini C.; Cimato A. & Heimler D. (2006). *Characterization of Violetto di Toscana, a typical Italian variety of artichoke (CynarascolymusL.).* J. Food Chem., Vol.95:221-225.

[18]. Bonnaillie C. ; Salacs M. ; Vassiliova E. & Saykova I. (2012*).* *Etude de l'extraction de composés phénoliques à partir de pellicules d'arachide (Arachishypogaea L.)*. Revue de génie industrie., Vol.7 :35-45.

[19]. Milane, H.(2004). La quercétine et ses dérivés : molécules à caractère prooxydant ou capteurs de radicaux libres'', études et applications thérapeutiques. Thèse de doctorat de l'université de Louis Pasteur., p.13-36.

[20]. Kansole.(2009). Etude ethnobotanique, phytochimique et activites Biologiques de quelques lamiaceae du burkinafaso: Cas de leucasartinicensis (Jacquin). The DEA., 78p.

[21]. Markham, K. R. (1982). Techniques of flavonoids identification. Ed Academic Press., p.6-10.

[22]. Dehpour, D A. A.; Ibrahimzadeh, M. A.; SeyedFazelet N.; Seyed Mohammad, N. (2009). Antioxydant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. *Grasas Y Aceites*., Vol. 60: 405-412.

[23]. Savova, M.; Kolusheva Stourza, T. A.; Seikova, I.(2007). The use of group contribution method for predicting the solubility of seed polyphenols of VitisVinifera L. *Journal of the UTCM.*, Vol.42:295-300.

[24]. Ebrahimzadeh, M. A.; Pourmmorad, F.; Hafezi, S.(2008). Antioxidant activities of Iranian. Corn silk. *Turkish journal of biology.*, n°32,:43-49.

[25].Abou, Z.; Nabiel, A.; Saleh, M .; Sabry, I.; Negoufo,W.; Mamdouh, M .(1987). Flavonoids Of *Artemisia judaica, A. Monosperma*And *A. Herba-Alba. Phythoterapy),* , 26(2): p.1059- 3064.

[26]. Khennouf, S.; Iratni, N.; Baghiani, A.; Harzallah; Arrar, L.(2010). Antioxidant and antibacterial activities of extracts from *Artemisia herbaalba*Asso. Leaves and some phenolic Compounds. *Journal of Medicinal Plants Research* ., 4(13),p. 1273-280.

[27]. Marcheix, J.J. ; Fleuriet, A . ; Jay-Allemand, C.(2005). Les composes phénoliques des végétaux: Un exemple de métabolites secondaires d'importance économique. PPUR., p.84-86.