AENSI Journals

Global journal of medicinal plant research

ISSN:2074-0883

Journal home page: http://www.aensiweb.com/GJMPR/

# First determination of phenolic compound concentration and antioxydant activity of *Agave americana* leaves extracts from different regions of Algeria (NW)

[1]Hannane Rahmani, [1]Fouzia Toumi Benali, [1]Fatiha Koudach, [1]Mustapha Mahmoud Dif, [2]Nabil Mekhfi, [3]Narimen Nouredine, [1]Faiza Moumen, [1]Mohamed Rahmani

[1]*Laboratoire d'écodeveloppement des espaces, faculté de science de la nature et de la vie, université de Djilali liabes, sidi bel abbes, Algerie*
[2]*Laboratoire de biodiversité végétale : conservation et valorisation, faculté de science de la nature et de la vie, université de Djilali liabes, sidi bel abbes, Algerie.*
[3]*Laboratoire de chimie macromoléculaire faculté des sciences, université de Djilali liabes, sidi bel abbes, Algerie.*

**ARTICLE INFO**

*Article history:*
*Received 22 December 2014*
*Received in revised form 3 January 2014*
*Accepted 15 January 2015*
*Available online 22 January*

*Keywords:*
*Agave americana, total phenols, total flavonoids, condensed tannins, antioxidant capacity.*

**ABSTRACT**

**Background:** *Agave americana* is medicinal plant that grows in different region of the western of Algeria which influence on the concentration of secondary metabolites affected by different ecological factor. **Objective:** in our study we determinate the phenolic compounds and evaluation of antioxidant capacity Agave *americana* leaves**. Results:** Our results confirm that Tessala region have a high phenolic compound concentration and the highest antioxidant activity. **Conclusion.** We can explain this results to a biotic stress of aridity which contribute to the highest concentration of secondary metabolites



**To Cite This Article:** Hannane Rahmani, Fouzia Toumi Benali, Fatiha Koudach, Mustapha Mahmoud Dif, Nabil Mekhfi, Narimen Nouredine, Faiza Moumen, Mohamed Rahmani., First determination of phenolic compound concentration and antioxydant activity of *Agave americana* leaves extracts from different regions of Algeria (NW). **Adv. in Nat. Appl. Sci.,** *3(3): 1-6, 2015*

## INTRODUCTION

Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins (Yu-Ling HO *et al.*, 2012) .In recent years, the extract of many plants have been screened for their antioxidant activities(El diwani *et al.*,2009).

The genus *Agave* includes 166 species and is the largest genus in the family agavaceae that consists of 9 genera and 293 species. (Good-Avila *et al.*, 2006) and occurs natively in the arid and tropical regions of the Western Hemisphere, particularly Mexico and Central America (Zou *et al.*, 2006), although some species can be found growing as introduced in several countries of African (Zwane *et al.*, 2010) and Asia (Chen *et al.*, 2009).several authors have described the use of agave as a good source of fibre, food and beverage. (Almaraz-Abarca *et al.*, 2009). *Agave spp*. have been used in the treatment of scabies, tumors, syphilis and dysentery, and as insecticides. (Zou *et al.*, 2006).

*Agave Americana* L, ( Figure1) commonly known as centaury plant, is one of the most popular and the most abundant varieties of agave species. (chattopadhyay and Khan, 2012), native to arid and semi arid region from the southern to the northern south of America. (faucan (1998-2004). It was introduced to Europe and Africa by Spanish and Portuguese. (Msahli *et al.*, 2007), where it naturalized rapidly, especially in the high arid region around the shores of the Mediterranean. (Lewin *et al.*, 1985), it is now cultivated worldwide as an ornamental plant. (chattopadhyay and Khan., 2012).

Centaury plant is one of the 500 more widely used medicinal plants in several countries. (Lozoya and Lozoya,1982), for its diverse range of properties like anti-inflammatory.(Peana *et al*, 1997 ;Monterrosas-Brisson *et al*, 2013), cytotoxic and antitumor activity. (ketan *et al.*, 2011), antioxidant (Ben hamissa *et al.*, 2012), antibacterial (Khan *et al.*, 2010), and it also showed an antifungal activity against the pathogenic microorganisms (Santos *et al.*, 2009), this activities can be attributed to the presence of different chemical constituents including many of saponins, some rang of flavonoids comprise homoisoflavanoid (Tinto *et al.*,

**Corresponding Author:** Hannane RAHMANI., Laboratoire d'écodeveloppement des espaces, faculté de science de la nature et de la vie, université de Djilali liabes, sidi bel abbes, Algerie.
E-mail: rahmani22h@gmail.com

**Global Journal of Medicinal Plant Research, 3(3) May 2015, Pages: 1-6**

2005), flavanols isolated from the flowers of this plant (Subramanian and Nair., 1970), latter Parmar *et al* (1992) were identified for the first time a complex flavanone from the somatic tissue of *Agave americana*.

The objectif of our study is to determinate and quantify the phenolic compounds present in the leaves of *agave americana* and to evaluate their antioxidant capacity.

## MATERIALS AND METHODS

*Plant material:*

In this work, the leaves of *agave Americana* L (figure1) was collected during the period of mars-juin 2014 from three different regions (Tessala (semiarid climat), kristel (wet climat) and boufatis (semiarid climat), with different geographic location situated in the north west of Algeria, freshly leaves were conserved at $4^0$C and the other were washed firstly with tap water and dried in the shade at room temperature for 20 days.

**Fig. a.1:** *Agave americana* of Kristel

**Fig. b.1:** *Agave americana* of region Boufatis region

**Fig. a.1:** *Agave americana* of Tessala region.

*Extraction procedure:*

Two milligrams of dried leaves were macerated with 20ml of 96% methanol using a mortar and pestle and were kept for 24h.the methanolic extract was filtered through whatman NO. 1 filter paper and kept in the dark at $4^0$C.

**Global Journal of Medicinal Plant Research, 3(3) May 2015, Pages: 1-6**

*Determination of total phenolics:*

The phenolic total (TPC) of plant extract was determined by spectrometric method using Folin-Ciocalteu according to (Benhammou *et al*., 2012) with some modification. Two hundred microliter of each sample was added in the test tube followed by 1ml of Folin-Ciocalteu diluted 10 times with distillated water and 0.8 ml of 7.5% sodium carbonate.

After 30 mn, absorbance was measured at 750nm using a spectrophotometer and a gallic acid as standard ($y = 0,0001 x + 0,135$, $R^2 = 0,991$.). .the results were expressed as gallic acid equivalents.

*Total flavonoids content:*

Total flavonoids in leaves were measured with aluminium chloride (AlCl3) with catechine as standard, according to ( kim *et al*., 2003) with a slight modification.

For the determination of total flavonoids (TF), an aliquot of the extract (500 µL) was added to 10 mL test tube containing 1.5mL of distilled water. 0.3 mL of 5% NaNO2 was added to each mixture and rested for 5 min before Addition of 0.3 mL 10% AlCl3. After 6 min, 2 mL of 1 M NaOH was added and votexed for 10 s. The absorbance of reaction was measured at 510 nm. Results were expressed as Catechin Equivalent (CE) mg/g dry weight by comparison with the catechine standard curve.( $y = 0,0021x + 0,0275$, $R^2 = 0,9917$)

The total flavonoids content was calculated as catéchine (mg CE/g dry weight) using the following formula based on the calibration curve.

*Condensed tannins content:*

Condensed tannins content was determined by using the vanillin-Hcl methode reported by (Price *et al*., 1978). Aliquots (0.1ml) of each extract were mixed with 3ml of 4% vanillin and 1.5 ml of Hcl reagent in test tube, incubated during 20mn at room temperature and the absorbance was read at 500nm.

Catechine was used as standard ($y = 0,0028x - 0,0253$, $R^2 = 0,9983$) and the results obtained were expressed as mg catechine equivalent/g dry weight.

*DPPH radical scavenging activity assay:*

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured according to (Wang *et al*., 1998). Briefly, volume of 50 µl of various concentrations of each sample is added to 1.950 ml of the methanol solution of DPPH (0.025 g / l) freshly prepared. With regard to the negative control, the latter is prepared by mixing 50 µl parallel methanol with 1.950 ml of a methanol solution of DPPH at the same concentration used. The reaction mixture was incubated in the dark for 30 and at room temperature and the absorabance was measured by UV spectrophotometry at 515nm.

The radical scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{\text{(Absorbance of control – Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

*Statistical study:*

All evaluations of results were performed twice. Data were expressed as means standard derivation (S.D.).

## RESULTS AND DISCUSSION

In the last few years, identification and extraction of phenolic compounds from different plants has become a major area of health and medical related research (Dai *et al*., 2010).the present study evaluates the phenolic, flavonoids content and condensed tannins as well as antioxydante capacity of dry sample of Agave *americana* leaves.

Phenolic compounds were extracted with methanol solvent for the fresh and dry sample. For this we have chosen 3 stations with different geographic locations, then we have determinate and analysed the concentration of total phenolic compounds in dried leaves of *A.americana* spectrophotometric methods .the results are presented in (Table1).

| | TPC (mg GAE/g) | | TFC(mg CE/g) | | TCT(mg GAE/g) | |
|---|---|---|---|---|---|---|
| | Dry matter | Extract | Dry matter | Extract | Dry matter | Extract |
| Tessala | 6.54±0.02 | 12,77±0.04 | 1.58±0.10 | 3.09±0.19 | 0.71±0.08 | 1.39±0.15 |
| Boufatis | 6.58±0.03 | 7.19±.0.04 | 2.88±0.05 | 3.15±0.06 | 0.81±0.02 | 1.22±0.01 |
| Kristel | 6.41±0.004 | 7.48±0.005 | 2.89±0.24 | 3.38±0.28 | 0.95±0.008 | 1.11±0.009 |

*Total phenolic content:*

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens (Rivzi and Pandey, 2009). In recent years, polyphenols have gained a lot of

importance because of their potential use as prophylactic and therapeutic agents in many diseases, and much work has been presented by the scientific community which focuses on their antioxidant effects (Vladimir-Knežević, 2012).

The total polyphenols content (TPC) was determined using the oldest methods by using the folin-ciocalteu and garlic acid as standard. The values were showed in (table 1). As shown in the table the TPC ranged from (6.41 mg GAE/g to 6.58 mg GAE/g) in dried samples while in the fresh mater ranged from (3.90 mg GAE/g to 5.87 mg GAE/g) .The highest concentration of total polyphenols was determined in dry mater of Boufatis region 6.58 mg GAE/g

Our results is similar of results (7.7 mg) reported by Nasri *et al.* (2012) and lower than the concentration (8.70 mg) reported by Ben hamissa *et al*. (2012).

*Total flavonoids content:*

Flavonoids are phenolic substances isolated from a wide range of vascular plants (Pietta, 2000), they have aroused considerable interest recently because of their potential beneficial effects on human health-they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities (Sharma *et al*., 2012). The yield of TFC was shown in table 1.

The dried leaves extracts of the two region Boufatis and Kristel showed the highest value of flavonoids content (2.88 mg CE/g and 2.89 mg CE/g) , while Tessala region showed the lowest value (1.58 mg CE/g).

The results our study is highest of the result obtained by Ben hamissa *et al. (2012).*

*Condensed tannins:*

Tannins are secondary metabolites that occur naturally in variety of plants (Iqbal *et al.,* 2011), they have a protective function in the bark of the roots and stems, or any outer layers of plants (Clinton, 2009). Condensed tannins content CT was determinate by vanillin essay and the result was presented in table1.

In our study we have found the higher concentration of CT in the dried extracts of Kristel locality (0.95 mg GAE/g), and the lower concentration the dried extracts of Tessala locality (0.71 mg GAE/g). When we compare our result with the research of Nasri *et al.,* 2012 we can say that our plant is more concentrated (0.95 mg GAE/g) than the concentration (0.4 mg GAE/g) obtained by Nasri *et al.,* 2012.

*DPPH radical scavenging activity:*

DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract (Sahu *et al.,* 2013) because it is fast, easy and reliable and does not require a special reaction and device (Aksoy et al .,2013).the $IC_{50}$ of metanolic extracts was represents in the table 2.

| Region | IC 50 (mg/ml) |
|---|---|
| Tessala | 179,310345 |
| Boufatis | 338,181818 |
| kristel | 342,857143 |

*Conclusion:*

The contents of total phenols, total flavonoids and condensed tannins of *Agave Americana* leaves are positively correlated with antioxidant activity. We can explain the highest concentration of phenolic compound in Tessala region to the highest aridity of this ecosystem compared to other region .

After we try to find the different between the molecules concentration using LC-MS to proof deeply these differences.

## REFERENCES

Aksoy, L., E. Kolay, Y. Agilonu, Z. Aslan and M. Kargioglu, 2013. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic Thermopsis turcica. Saudi Journal of Biological Sciences, 20: 235-239.

Almaraz-Abarca, N., E.A. Delgado-Alvarado, V. Hernández-Vargas, M. Ortega-Chávez, G. Orea-Lara, A. Cifuentes-Díaz de León, J. A. Ávila-Reyes y R. Muñiz-Martínez, 2009. Profiling of phenolic compounds of somatic and reproductive tissues of *Agave durangensis* Gentry (Agavaceae). Am. J. Appl. Sci., 6: 1076-1085.

Ben hamissa A., M. Seffen, B. Aliakbarian, A. Casazza, P. Perego, A. Converti, 2012. Phenolics extraction from Agave americana (L.) leaves using high-temperature, high-pressure reactor. *Food and Bioproducts Processing*, 90(1): 17-21.

Benhammou, N., N. Atik Bekkara, T. Kadifkova Panovska, 2006. Antioxidant activity of methanolic extracts and some bioactive compounds of Atriplex halimus-C. *R. Chimie.,* 12: 1259-1266.

Chattopadhyay, D.P., J.S. Khan, 2012. Agave americana: A new source of textile fiber.Textile Potpourri.

Chen, P.Y., Y.C. Kuo, C.H. Chen, Y.H. Kuo, C.K. Lee, 2009 .Isolation and immunomodulatory effect of homoisoflavones and flavones from *Agave sisalana* Perrine ex Engelm.Molecules., 14: 1789-1795.

Clinton, C., 2009. Plant tannins: A novel approach to the treatment of ulcerative colitis. Natural Medicine Journal 1(3).

Dai, J., J. Russell, 2010. Plant Phenolics: Extraction, Analysis and Their Antioxidant Anticancer Properties. *Molecule*, 15: 7313-7352; doi:10.3390/molecules15107313

El Diwani, G., Sh. EL Rafie, S. Hawash, 2009. Antioxydant activity of extracts obtained from residues of nodes leaves stem and roots of Egyptian Jatropa curcas.african journal of pharmacy and pharmacology, 3(11): 521-530.

Faucan, P., (1998-2005) http://www.desert-tropicals.com/Plants/Agavaceae/Agave_americana.html.Acccssed 17 September 2014

Good-Avila, S.V., V. Souza, B.S. Gaut, L.E. Eguiarte, 2006. Timing and rate of speciation in *Agave* (Agavaceae). *Proc. Natl Acad. Sci. USA.* 103: 9124-9129. doi:10.1073/pnas.0603312103.

Iqbal, Z., M.S., Sajid, R. Zahid Abbras, Z. Ud Din Sindhu, 2010. Determination of Condensed Tannin Contents from Different Plants of Kherimurat Rangeland (Attock, Pakistan). J. Agric. Soc. Sci., 7: 3.

Ketan, V.K., D. Harikesh, R. Chandrashekhar, Tenpe, 2011. Anticancer activity of the ethanolic extracts of agave Americana leaves. Pharmacologyonline., 2: 53-68.

Khan, M.T.J., K. Ahmad, M.N. Alvi, N. Amin, B. Mansoor, M. Asif, F.Z. Khan, M. Jamshaid, 2010. Antibacterial and Irritant Activities of Organic Solvent Extracts of *Agave americana* Linn., *Albizzia lebbek* Benth. *Achyranthes aspera* Linn. and *Abutilon indicum* Linn - A Preliminary Investigation. Pakistan J. Zool., 42(1): 93-97.

Kim, D., S.W. Jeong, C.Y. Lee, 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*, 81: 321-326.

Lewin, M., and EM. Pearce, 1985. Handbook of fiber sciences and technology: vol IV, Fiber chemistry,New York: Marcel-Decker-inc.

Lozoya, X., M. Lozoya, 1982. *Flora Medicinal de México. Primera Parte: Plantas Indígena* IMSS:D.F., México.

Monterrosas-Brisson, N., M.L. Arenas-Ocampo, E. Jiménez-Ferrer, A.R. Jiménez-Aparicio, A. Zamilpa, M. Gonzalez-Cortasar, J. Tortoriello, M. Herrera-Ruiz, 2013. Anti-inflammatory activity of different Agave plants and the compound Cantalasaponin-1. Molecules, 18(7): 8136-8146.

Msahli, S., Y. Chaabouni, F. Salki and Y.J. Drean, 2007. Mechanical behavior of Agave *Americana* L. Fibres: correlation between fine structure and mechanical propreties. Journal of Applied Sciences, 7(24): 3951-3957.

Nasri, H., H. Ben Salem, 2012. Effect oforaladministrationof Agave americana or Quillaja saponaria extracts on digestion and growth of Barbarine female lamb.Livestock Science, 147: 59–65.

Pandey, K and S.I., Rizvi., 2012. Recent Advances in Health Promoting Effect of Dietary Polyphenols. Curr. Nutr. Food. Sci., 8: 254-264.

Parmar, V.S., H.N. Jha, A.K. Gupta, A.K. Prasad, 1992. Agamanone, a flavanone from *Agave Americana*. Phytochemistry, 31: 7 2567-2568.

Peana, A.T., M.D.L. Moretti, V. Manconi, G. Desole, P. Pippia, 1997. Anti-inflammatory activity of aqueous extracts and steroidal sapogenins of *Agave americana*. Planta Med., 63: 199-202.

Pietta, P.G., 2000. J. Nat. Prod., 63(7): 1035-1042.

Price, M.L., S. Van Scoyoc and L.G. Butler, 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem., 26: 1214-1218.

Quézel, P and S. Santa, 1963. Nouvelle flore de l'Algérie et des régions désertiques méridionales. C.N.R.S., Paris.

Sahu, R.K., M. Kar and R. Routray, 2013. DPPH free radical scavenging activity of some leafy vegetables used by tribals of Odisha, India. J. Med. Plants Stud., 1: 21-27.

Santos, J.D.G., A. Branco, A.F. Silva, C.S.R. Pinheiro, A.G. Neto, A.P.T. Uetanabaro, S.R.O.D. Queiroz, J.T.A. Osuna, 2009. Antimicrobial activity of Agave sisalana. Afr. J. Biotechnol., 8: 6181-6184.

Sharma, T., L. Joseph, S. Khah, 2012. Ethanobotanical Claims and Pharmacological Activities of *Cuscuta reflexa Roxb*.- A Review. Research Gate: Pharmaceutical Sciences, 1: 15-18.

Subramanian, S.S. and A.G.R., Nair, 1970. Chlorogenein and Kaempferol Glycosides from the Flowers of Agave amaricana, Phytochemistry, 9(12): 2582. doi:10.1016/S0031-9422(00)85782-9

Tinto, W.F., J.L. Simmons-Boyce, S. McLean, et al, 2005. Constituents of Agave americana and Agave barbadensis.Fitoterapia; 76: 594-7.

Yu-Ling, H.O., S.S. HUANG, J.S. DENG, Y.H. LIN, Y.S. CHANG and G.J. HUANG, 2012. In vitro antioxidant properties and total phenolic contents of wetland medicinal plants in Taiwan Botanical Studies., 53: 55-66.

Vladimir-Knežević, S., B. Blaženković, M. Bival Štefan, A. Alegro, T. Kőszegi and J. Petrik, 2011. Antioxidant activities and polyphenolic contents of three selected *Micromeria* species from Croatia. *Molecules*, 16(2): 1454-1470, ISSN 1420-3049

Wang, M., J. Li. Rangarajan, Y. Shao, E.J. LaVoie, T.C. Huang and C.T. Ho, 1998. Antioxidative Phenolic Compounds from Sage (Salvia officinalis). J. Agic. Food Chem., 46s: 4869-4873.

Zou, P., J. Fu, HS. Yu, J. Zhang, L.P. Kang, Ma BP, XZ. Yan, 2006. The NMR studies on two new furostanol saponins from Agave sisalana leaves. Mag Reson Chem., 21: 1397-1405.

Zwane, P.E., M.T. Masarirambi, N.T. Nagagula, A.M. Dlamini and E. Bhebhe, 2011. Exploitation of *Agave americana* L. Plant for Food Security in Swaziland, American Journal of Food and Nutrition, 1(2): 82-88. doi:10.5251/abjna.2011.1.2.82.88.