Heterocyclic Letters
Vol. 12| No.1|25-40|November -January|2022
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

IN VITRO ANTIOXIDANT ACTIVITY OF DIFFERENT POLARITY EXTRACTS AND METABOLITES ISOLATED FROM *MOLTKIA CILIATA* GROWING IN ALGERIA

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ABSTRACT

The plant *Moltkia ciliata* belong to a family *Boraginaceae***,** although this plant has been traditionally used for the treatment of various ailments still no systematic pharmacognostical, phytochemical and pharmacological work has ever been carried out on this potential plant. In the present study, we reported the evaluation of antioxidant properties using electrochemical and spectrophotometrical assays and determination *of total bioactive compounds* content of the ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) extracts of *Moltkia ciliata* growing in Algeria. The results showed that EtOAc extract which had the highest level of *bioactive compounds* content, exhibited the most potent antioxidant capacity in each assays compared to the *n*-BuOH extract. Chromatographic and HPLC analysis of extract shows many compounds with different quantities. This study suggested that the differences of the potency of the antioxidant activity may be explained by the differences in the polyphenol and flavonoid levels. The study concludes this plant is a rich source of phenols and flavonoids, and also showed good in-vitro antioxidant activity by all methods. Thus, the plant *M. ciliata* can be explored as a potential source of natural antioxidant.

Keywords*: Moltkia ciliata, bioactive compounds*, ***A***ntioxidant capacity; HPLC; TLC.

1. INTRODUCTION

Many synthetic drugs, such as butyl hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) are commonly used as antioxidants, but have recently been found to cause many serious diseases, the consumption of plant foods and natural antioxidant supplements can protect the body from various diseases, including cancer, cardiovascular disease and neurodegenerative diseases **[i].** Recent studies have shown that secondary plant metabolites can be a source of medicinal agents **[ii, iii]**. It is expected that phytochemicals with antibacterial activity will be used in the treatment of bacterial, fungal and viral infections **[iv]**.

To develop natural antioxidants for food, cosmetics and others, aromatic antioxidants have been studied. The plant is rich in phenolic compounds, whose type and content are fundamentally different between different parts of the plant **[v]**. Polyphenols play diverse roles such as functioning as antioxidant, antimicrobial, anti-allergic, anti-inflammatory and anticancer agents **[vi]**. These compounds possess low molecular weight and are biologically active and are responsible for antioxidant properties **[vii]**. Previous research reported that phenolic compounds, tannins, anthocyanins, and flavonoids play a role in inhibiting free radical removal through various mechanisms **[viii].** This work was devoted to the chemical, antioxidant study of organic extracts of *Moltkia ciliata*, collected using the mixture (methanol/water) applying different analytical methodologies.

2.MATERIALS AND METHODS

1.1Chemicals and reagents

All chemicals, solvents and reagents were analytical grades or purest quality purchased and all used as received.

2. 2. Plant Material

Moltkia ciliata (Forsk) of the Boraginaceae family is the most abundant of the nine known *Moltkia* species.

The Boraginaceae family consists of about 131 genera, most of which are annual, semi-annual or perennial herbs, sub-shrubs, and the plants of this family are widespread in tropical and subtropical regions. The members of the family grow mainly on dry, cliffy and sunny habitats **[ix].** *M. ciliata* is a small, long-stemmed shrub that is wrapped with hard bristles, no longer than 30 cm long. The old stems are smooth white, while the new ones are brown. The leaves are placed on the stem without necks of 2-3 cm, wide spread, germinate in hard ground as well as sandy and spread nipple in all Gulf countries as well as in Egypt, Iraq, Iran and Palestine **[x].** This plant is a food sanctuary for some desert animals especially camel; it has several traditional uses in the medical field used for abdominal diseases, wound healing and treatment against scorpion stings. *M. ciliata* has attracted the interest of many researchers, especially in the statistical studies of some characteristics of desert plants **[xi]**.

Figure 1: Geographical distribution of the plant in Africa in 2007 and image showing the outside of the plant *Moltkia ciliata*

2.3. The phytochemical study

2.3.1. Phytochemical Screening

To determine and limit the different active substances present in the aerial part of the plant, we performed many preliminary phytochemical tests **[xii-xiv]**.

2.3.2. Extraction and isolation

The aerial part (100g) of the plant were macerated at room temperature with MeOH/H_2O (80:20 v/v) (99%; Biochem Chemopharma (Canada)) for 24 h, three times. After filtration, the filtrates were combined, concentrated in vacuum up to 35°C and dissolved in distilled H_2O at a rate of 50 ml per 100 g of dry matter, under magnetic stirring and then put at the refrigerator for one night. After filtration, the solution was subjected to successive liquid-liquid type extractions

using solvents of increasing polarity, starting with petroleum ether, then chloroform, then ethyl acetate and finally with n-butanol, each extraction is repeated three times except with ethyl acetate, the organic phases were dried with magnesium sulfate anhydrous (97%, Acros Organics), filtered using filter paper and concentrated in vacuum 35°C.

2.3.3. Chromatographic analysis (thin layer chromatography)

Thin Layer Chromatography (TLC) is a rapid analytical technique and simple, used for the purpose of separation and identification of metabolites. TLC is mainly based on adsorption phenomena: the sample containing one or more compounds is adsorbed on a stationary phase, and then is desorbed by a mobile phase (solvent or mixture of solvent). The different compounds in the sample are separated according to their adsorption rates-desorption, this technique provides an overview of the metabolites present in an extract **[xv].**

2.3.4. Phenolic identification

The composition of the extracts was analyzed by high performance liquid chromatography (HPLC) **[**xvi**]**. We used a Shimadzu (LC 20, Japan). System comprised of a LC-10AD pump, a CTO-10A column oven, a SPD-10A UV-DAD detector, a CBM-10A interface and a LC-10 Workstation was utilized. A LC-18 column (250 mm x 4 mm i.d. x 5 mm) was employed. Samples were injected. The components of the samples were separated by gradient elution at 30 °C. The mobile phases were: A, 98: 2 (v/v) acetic acid, and B, acetonitrile and the elution gradient was: 0–5 min, 5% B; 10 min, 10% B; 11 min, 20% B; 20 min 20% B; 30 min 40% B; 40 min 50% B; 50 min 80% B. The flow rate was 0.8 ml/min and the detection wavelength was 285 nm. Phenolic compound standards gallic acid, ascorbic acid, querecetin, chlorogenic acid, vanillin, caffeic acid and rutin were dissolved in solvents extraction and used for identification of the phenolic acids present in different extracts of *M. ciliata*. Peak identification in HPLC analysis was achieved by comparison of retention time and UV spectra of reference standards. Quantification of individual phenolic compounds in the extracts was done using the peak area of reference compounds and reported as mg/g of extract.

2.4. Determination of total bioactive compounds content

2.4.1. Determination of total phenolic content

The total concentrations of phenolic content of extracts were determined using the Folin-Ciocalteu Reagent (FCR; 98%; biochem chemopharma (Canada)) according to the method described by (Singleton Rossi 1999) **[iiix]**, where we use the gallic acid ($C_6H_2(OH)_3COOH$; 99%; Alfa Aesar (Germany)) as reference phenol. 1ml of extract solution add 0.5mL of dilute Folin ciocalteu reagent 10 times, after 5 min add 2 ml of sodium carbonate solution (Na_2CO_3, 7.5%) the mixture completed the reaction for 30 min at room temperature. The absorbance was read at 765 nm using a UV-Vis spectrophotometer; experiments were performed using a UV-1800 UV-Vis spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) at a wavelength of 400 to 800 nm. The total phenolic content was calculated from the calibration curve Figure 2 and the results were expressed as mg of gallic acid equivalent per g dry weight of extract **[viii, xvii]**.

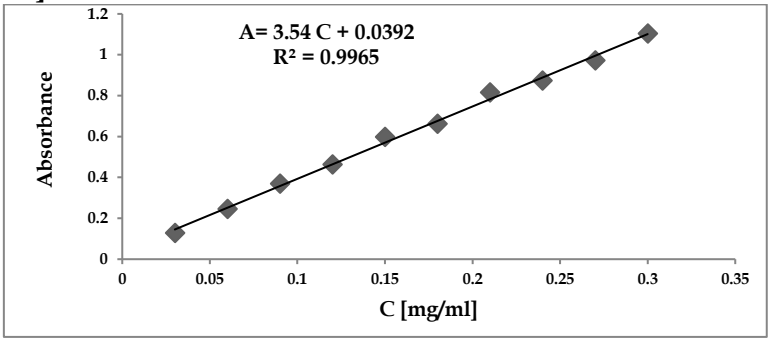

Figure 2: Standard curve of Gallic Acid

2.4.2. Determination of total flavonoids content

The total flavonoids content were determined using the method of (Ordonez *et al* 2006) **[xviii],** where we use the rutin ($C_{27}H_{30}O_{16}$; 97 %; Alfa Aesar (Germany)) as reference flavonoid**.** A volume of 0.5 ml of 2% $AlCl_3$ methanol solution was added to 0.5 ml of extract solution. The mixture was incubated for 1 h at room temperature for yellow color appearance; the absorbance was measured at 420 nm. The total flavonoids content was calculated from the calibration curve Figure 3, and the results were expressed as mg of routine equivalent per g dry weight of extract **[**xix**].**

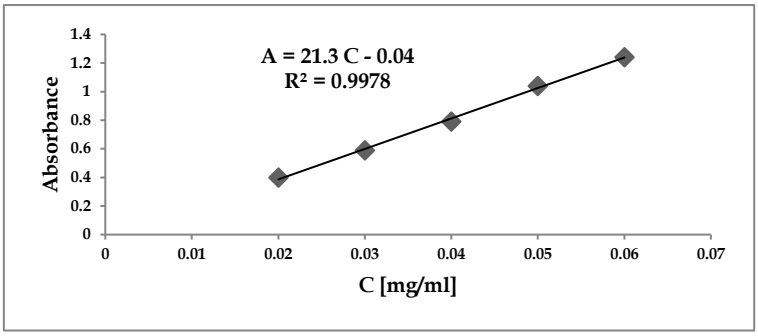

Figure 3: Standard curve of Routine

2.4.3. Total flavanol content

The total flavonols content were determined using the method of Kumaran and Karunakaran (2007) **[xx],** where we use the quercetin ($C_{15}H_{10}O_7$; 97%; Alfa Aesar (Germany)) as reference flavanol. 2.0 ml of the sample was mixed with 2.0 ml of 2% $AlCl_3$ prepared in methanol and 3.0 ml of 50 g/l sodium acetate solution were added. The mixture was incubated at 20°C for 2.5 h after which the absorption was read at 440 nm using spectrophotometer. The total flavanol content was calculated from the calibration curve Figure 4, and the results were expressed as mg of quercetin equivalent per g dry weight of extract **[**xxi**].** The total experiment was conducted in triplicate and the results were expressed as means ± SD (standard deviation).

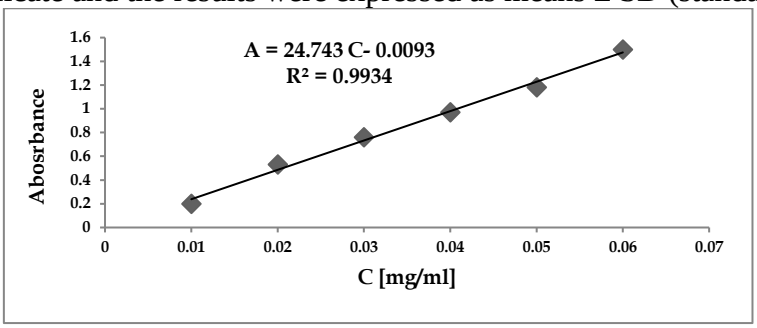

Figure 4: Standard curve of Quercetin

2.5. Antioxidant activities

2.5.1. Free radical scavenging activity

2.5.1.1. Free 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical-scavenging ability

Free radical scavenging activity of extracts were measured using a stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) ($C_{18}H_{12}N_5O_6$;97(%); Sigma-Aldrich Chemicals (St Louis, MO, USA)) and was determined spectrophotometrically by the slightly modified method of Djeridane and al. (2010) **[**xxii**].** The extracts with potential antioxidant activity scavenge the initial violet color of free DPPH radicals and turn it into yellow color. This change in color is proportional to the radical scavenging activity. Briefly, the assay contained 1.5 ml of 100 µM DPPH in methanol and 1.5 ml of various concentrations of extracts solution and standards in

the same solvent. The contents were mixed well immediately and then incubated for 30 min at room temperature (28 ± 1)°C. The degree of reduction of absorbance was recorded in UV–Vis spectrophotometer at 517 nm. All data were analyzed and expressed as means ± standard deviation (in three replications, n = 3).

The ability of the test sample to quench 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) (% Inhibition of DPPH) was calculated using the following equation (1)

$$\textbf{DPPH scavenging activity}\ (\%) = \frac{\textbf{Absorbance of control} - \textbf{Absorbance of sample}}{\textbf{Absorbance of control}} \times \mathbf{100} \quad (\mathbf{1})$$

The activity is expressed as inhibitory concentration IC_{50}, which is the amount of antioxidant necessary to decrease by 50% the initial DPPH concentration **[**xxiii**,**xxiv**].**

2.5.1.2. Free superoxide anion radical ($O_2^{\cdot-}$) radical-scavenging ability

The measurement of the antioxidant capacity of the studied extracts was performed using an electrochemical method based on cyclic voltammetry techniques **[**xxv**,**xxvi**]** Cyclic voltammetry measurements were performed using PGP301 potentiostat (radiometer analytical SAS) connected to an electrochemical cell of 25 ml and conventional three electrode system was employed. Glassy Carbon (GC) working electrode (radiometer analytical SAS) is having diameter 3 mm, a polishing of the working electrode using p4000 sand paper is performed before each electrochemical test, an auxiliary platinum electrode with a diameter of 0.5cm and an Hg/Hg_2Cl_2 reference electrode (3.0 M KCl). The antioxidant capacity was obtained using the current density of the anodic curve of the voltammogram **[**xxvi**,** xxvii**]**. Data acquisitions were accomplished with a Pentium IV (CPU 4.0 GHz and RAM 2 Gb) microcomputer using VoltaMaster4 software version 7.08 (radiometer analytical SAS). Graphs plot and calculus were carried out using Origin Lab software version 2.0 (Integral Software, France). The superoxide anion radical was generated by one electron reduction of the commercial molecular oxygen (O2) dissolved in DMF (analytical grade, Sigma-Aldrich) containing 0.1 M TNBHFP at room temperature (28±1) °C. The scan rate was kept at 100 mV/s and potential window was from -1.6 to -0.0 V, the solutions were saturated with high purity commercial oxygen for 10 min prior to each experiment. Quercetin was used as a standard in the calculation of antioxidant capacity of the studied samples. The calibration graph is obtained by plotting the current density of the anodic curve of the voltammograms of each sample of the standard versus its concentration. The ability of the test sample to quench superoxide anion radicals ($O_2^{\cdot-}$) (% Inhibition of $O_2^{\cdot-}$) was calculated using the following equation (2) **[**xxviii**,** xxix**],** and according to the researcher's method Bourvellec et al. **[xxx].**

$$\mathbf{O_2^{\cdot-}\ radical\ scavenging\ activity}\ \% = \frac{\mathbf{i_0} - \mathbf{i_s}}{\mathbf{i_0}} \times \mathbf{100} \quad (\mathbf{2})$$

Where i_0 and i_s are the anodic peak current densities of the superoxide anion radical in the absence and in the presence of the extract.

2.5.2. The ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to the method of Benzie and Strain (1996) with slightly modification **[**xxxi**].** The stock solutions included 300mM acetate buffer (3.1 g $C_2H_3NaO_2$-$3H_2O$ and 16ml $C_2H_4O_2$), pH 3.6, 10mM TPTZ (2, 4, 6-tripyridyl-s-triazine; $C_{18}H_{12}N_6$;98(%); Sigma-Aldrich Chemicals (St Louis, MO, USA)) solution in 40mM HCl, and 20Mm $FeCl_3$-$6H_2O$ solution. The FRAP solution was prepared by mixing 25ml acetate buffer, 2.5ml TPTZ solution, and 2.5ml $FeCl_3$-$6H_2O$. The ascorbic acid ($C_6H_8O_6$; (99%); Alfa Aesar (Germany)) used a standard compound and at differing concentrations (0.1 to 0.6mg/ml). 0.3 ml of ascorbic acid solutions was mixed with 0.27 ml of the FRAP solution. The mixture was Leave in the dark for 30 min and gets a violet color. The absorbance was measured at 593 nm in a spectrophotometer. All determinations were carried out in triplicate. Using the standard curve

relationship of ascorbic acid Figure 5 , we evaluated the antioxidant activity of organic extracts and the equivalent amount of 1 g of the extract, this was calculated the following equation (3) **[**xxxii**]:**

$$\textbf{FRAP value} = \frac{\mathbf{A_{593nm}\ of\ test\ sample(conc.\ 1000\ (\mu g/ml)) \times FeCl_3 concetration(\mu mol/l)}}{\mathbf{4min\ A_{593nm}\ of\ FeCl_3(at\ conc.\ 1000(\mu g/ml))}} \quad (3)$$

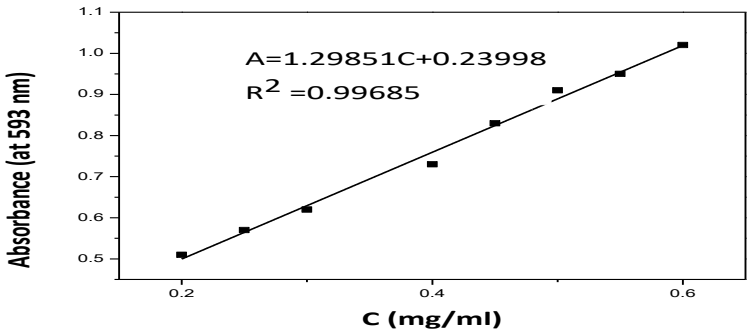

Figure 5: Standard curve of ascorbic acid (FRAP assay)

2.5.3. Total antioxidant capacity (Molybdate ion reduction)

The total antioxidant capacity of the extracts was evaluated by phosphomolybdenum method **[**xxxiii**]**. This method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of green Mo (V) complexes with a maximal absorption at 695 nm **[**xxxiv**].** The ascorbic acid used a standard compound and at differing concentrations (0.01 to 0.2 mg/ml). The reagent solution was prepared by mixing (sulphuric acid 0.6M, sodium phosphate 28 mM (Rathburn Chemical (Walkerburn, Peebleshire, UK)), ammonium molybdate 4mM ($(NH_4)_2MoO_4$; (98%); BioChem (Quebec, Canada)). 0.3 ml of ascorbic acid solutions was mixed with 3 ml of the detergent solution, leave it for an hour in a water bath temperature of 95^0C, we get the green color **[xxxv].** Using the standard curve relationship of ascorbic acid Figure 6 , we evaluated the antioxidant activity of organic extracts and the results were expressed as ascorbic acid equivalent (AAE) in milligrams per gram dry extract.

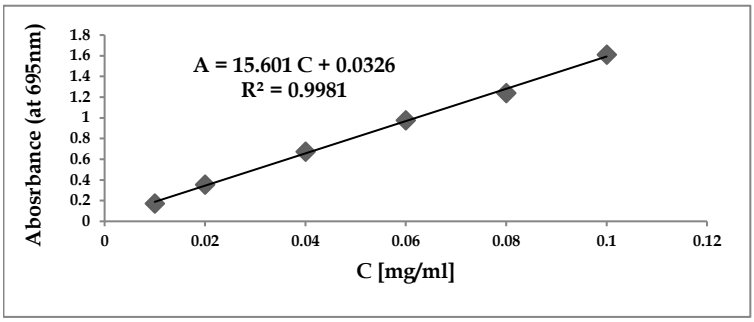

Figure 6: Standard curve of ascorbic acid (TAC assay)

2.5.4. ABTS Radical Cation Decolorization Assay

The cation radical scavenging activities of the extracts were determined using ABTS 2,2´-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt ($C_{18}H_{24}N_6O_6S_4$;98(%); Sigma-Aldrich Chemicals (St Louis, MO, USA)) (Pellegrini and al., 1999) **[**xxxvi**]**. This method is based on the decolorization of blue–green color at 734 nm, since ABTS radical is scavenged. $ABTS^{\cdot+}$ is a stable blue–green chromophore radical cation characterized by an

absorption band at about 734 nm which losses its color in the presence of an antioxidant molecule **[**xxxvii**]**. For ABTS assay, the stock solutions included 7mM $ABTS^+$ solution and 2.4mM potassium persulphate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1ml $ABTS^+$ solution with 60 ml methanol to obtain an absorbance of (0.706 ± 0.001) units at 734 nm using the spectrophotometer. Fresh $ABTS^+$ solution was then prepared for each assay. Plant extracts/standard (BHT; butylated hydroxytoluene; 99(%); Sigma-Aldrich Chemicals (St Louis, MO, USA)) (1ml) of different concentration was allowed to react with 1ml of the $ABTS^+$ solution freshly prepared solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer **[**xxxv**]**.
The ABTS scavenging capacity of the extracts was compared with that of standard and percentage inhibition calculated using the following formula**[**xxxviii**].**

$$\textbf{ABTS radical scavenging activity}\ (\%) = \frac{\mathbf{Abs_{control} - Abs_{sample}}}{\mathbf{Abs_{control}}} \times \mathbf{100} \qquad (\mathbf{4})$$

$Abs_{control}$: The absorbance of ABTS radical + methanol. **Abs_{sample}: The absorbance of ABTS radical + extracts/standard.**

Statistical Analysis

Experimental values are given as means ± standard deviation (SD) of three replicates for antioxidant activity. Statistical calculations were carried out by OriginPro Version 9.1 software (OriginLab Corporation). Values of $p < 0.05$ were regarded as significant.

3. RESULTS AND DISCUSSION

To support our development to study the antioxidant efficacy through lists of several chemical compounds in the organic extracts of *M. ciliata* we have therefore used several methods.

3.1. Phytochemical study

3.1.1. Preliminary phytochemical

The photochemical screening studies of the aerial part of *M. ciliata* are shown in Table 1. The obtained results showed that this plant rich in natural compounds: flavonoids, alkaloids, Sterols, Volatile oils, saponins and tannins.

Table 1: Phytochemical constituents of *M. ciliata*: (+++): important Presence, (++): average Presence, (+): weak, presence and (-): absence.

Compounds	Results
Flavonoids	**(+++) Appearance of a red color characteristic aglycone flavones**
Alkaloids	**(++) appearance of turbidity**
Sterols	**color changes from purple to blue or green (++)**
Volatile oils	**(+) A distinctive odor emission**
Saponosides	**(++) Appearance of foam shaken in for15 min**
Tannins	**(+++) Appearance of a blue-green color**
Coumarins	**(-) no precipitate**
Cardenolides	**(-) no precipitate**

3.1.2. Extraction yield

It is calculated by the following equation (5),

$$\mathbf{R}\% = (\mathbf{m_f} / \mathbf{m_i}) \times \mathbf{100} \qquad (\mathbf{5})$$

Where: m_f; The mass of the organic extract obtained and m_i; The mass of the plant sample

Note the difference in extraction yield between organic extracts, the separation of components depends on the polarity of both solvent and component **[**xxxix**].** The extracts AcOEt and *n*-BuOH are of the highest value, where the study was confined around them.

3.1.3. HPLC analysis

All the standards were separated within 30 min and showed good resolution between analyte peaks. Figure 7 shows the chromatogram of mixed standard solution.

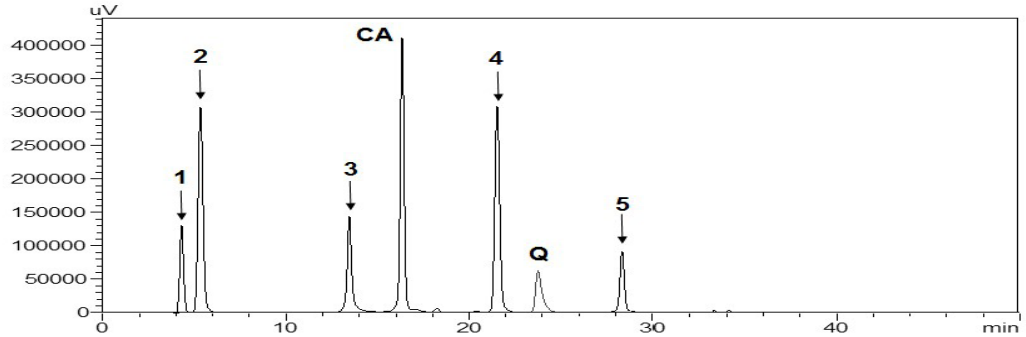

Figure 7: Chromatograms of standard phenolic compounds; 1. Ascorbic acid; 2.Gallic acid; 3. Chlorogenic acid; CA. Caffeic acid;
4. Vanillin; Q. Quercetin; 5. Rutin

The calibration curve of the individual phenolic compound was based on these five concentrations of standard solutions. The peak area values were the average values of three replicate injections. The results of calibration equations and correlation coefficients are summarized in Table 2, and a good correlation was found between the peak area (y) and the concentrations (x) ($R^2 > 0.995$) for all the compounds in the range of concentration tested at 300 nm.

Table 2: Calibration equations and regression coefficients for all standards

Standard	Retention time[min]	Calibration equation	Regression coefficient(R^2)
Ascorbic acid	**4.23**	**y = 2851.73x+212**	**R^2 = 0.998**
Gallic acid	**5.29**	**y = 23616x-723**	**R^2 = 0.998**
Chlorogenic acid	**13.39**	**y = 39775.06x-188**	**R^2 = 0.999**
Vanillin	**21.46**	**y = 80555.42x +321**	**R^2 = 0.989**
Rutin	**28.37**	**y = 31189.46+184x**	**R^2 = 0.988**
Quercetin	**24.04**	**y = 71439.77x+182**	**R^2 = 0.989**
Caffeic acid	**16.27**	**y = 70429.77x+102**	**R^2 = 0.999**

The constituents in the different extracts were analyses by HPLC. The contents of these components in different extracts were determined according to the calibration curves, where y was the peak area and x was the concentration of analyte (0–80 µg/ml). The quantitative results are summarized in Figure 8.

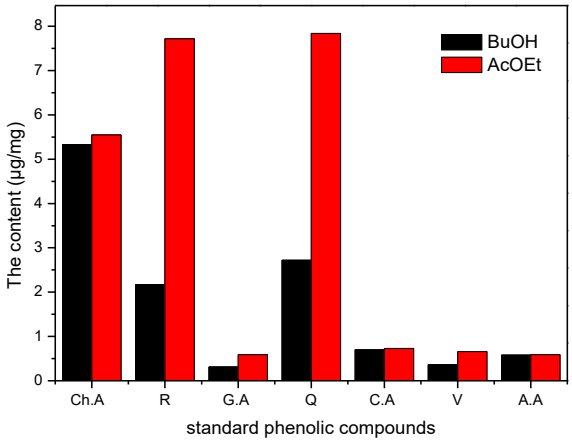

Figure 8: Constituents content analyses by HPLC, A.A: Ascorbic Acid; G.A: Gallic Acid; Ch.A: Chlorogenic Acid; C.A: Caffeic Acid; V: Vanillin; Q: Quercetin; R: Rutin

As shown, all compounds are present in the extracts with different quantities. Rutin, caffeic acid, quercetin and chlorogenic acid are present in significant quantities. Ethyl acetate extract is richer in compounds than others; this is due to the difference of these compounds in terms of polarity.

3.1.4. Chromatographic separation

Chemical constituents of the extracts were separated on aluminum-backed thin layer chromatography (TLC) plates (Merck, silica gel 60, F_{254}). The various extracts obtained were analyzed by layer chromatography thin from the solvent system: (methanol/chloroform/eau; 5/20/0.5), (methanol/chloroform; 1/3), (methanol/chloroform/n-butanol; 1/1/1) in order to separate and identify the polyphenols and flavonoids present in extracts. Separated chemical compounds were examined firstly using UV- 254nm and 365nm to detect florescent zones. After that, Separated chemical compounds sprayed with spray reagents in order to reveal spots. The possibility of the following types of compounds has been found **[**xxxx**,**xxxxi**]:**

- Purple: Flavon or flavonol containing OH at position C_5 and OH at position C_4 replaced or deleted.
- Brown: Some flavonoids contain OH in position C_5 or chalcones containing OH in position C_4 and lack OH on the aromatic ring B.
- Radiant blue: Isoflavones do not contain OH at position C_5 free.
- Yellow: Flavonol containing OH at position C_3 with or no free OH at position C_5.Where 7 compounds were identified in (*n*-BuOH) extract, 11 compounds in (AcOEt) extract.

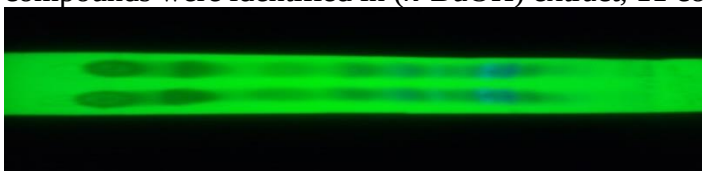

Figure 9: Results of TLC screening plates of *M. Ciliata* extracts, *n*-BuOH extract to the right and AcOEt extract to the left.

3.2. Total bioactive compounds content

Extraction is an important step for obtaining extracts with acceptable phytochemical concentration and strong antioxidant activity. The selection of extraction conditions is very important when we research natural compounds **[**xxxxii**].** Figure 10 represent the analytical data for phenolics content, flavonoids and flavanol of the extracts of *M. ciliata*. In comparison, AcOEt extract showed the higher quantities compared to n-BuOH extract. The AcOEt extract contains a large number of polyphenolic compounds and flavonoids mainly due to the polarity

of polyphenols determined **[**xxxxiii**]**. This compounds are important of this plant and due to the presence of these valuable constituents, it can have some pharmacological effects or/and can be beneficial for human consumption **[**xxxxiv**,** xxxxv**]**. These phytochemical compounds are known to be bioactive compounds and all play a role for antioxidant activity of *M. ciliata* extracts.

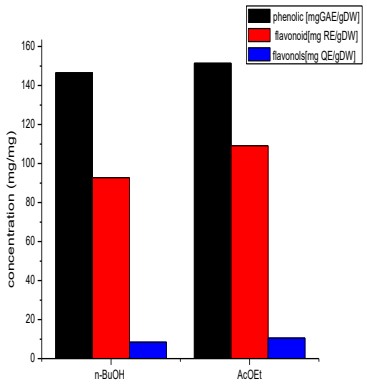

Figure 10: Total phenolic content, flavonoid and flavonols of extracts from *M. ciliata*

3.3. Antioxidant activity

Various methods were used to measure the antioxidant capacity of the studied extracts and the results obtained are shown in Table 3.

Table 3**: Antioxidant activity of *M. Ciliata* extracts as determined by the DPPH, FRAP, TAC, superoxide anion($O_2^{\cdot-}$), ABTS assays**

Extracts	IC_{50} DPPH (mg/ml)	FRAP Value (mg /g)	IC_{50} superoxide anion($O_2^{\cdot-}$) (mg/ml)	IC_{50} ABTS (mg/ml)	TAC Value (mg /g)
Standard	**0.28** ± **0.03**	**-**	0.23±**0.01**	**0.093±0.02**	**-**
AcOEt	**0.08 ± 0.02**	**272.09 ± 0.01**	0.79±0.04	0.19±0.05	**84.03 ± 0.08**
***n*-BuOH**	**0.14 ± 0.02**	**233.66 ± 0.07**	1.16±0.05	0.33±**0.04**	**73.61 ± 0.08**

3.3.1. Free radical scavenging activities study

The antioxidant capacity of the *M. Ciliata* extracts was expressed as IC50. The IC50 value was defined as the concentration (mg/ml) of samples that inhibits the formation of DPPH or ($O_2^{\cdot-}$) radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations and the results obtained are shown in Table 5.

3.3.1. a. Free 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical-scavenging ability

The DPPH radical scavenging ability of plant extracts and ascorbic acid standard shown in Figure 11. We note that increasing the concentration leads to an increase in inhibition, it is clear from the results obtained that the **AcOEt extract** shows the highest DPPH radical scavenging activity (0.20±0.02) which was significantly closer to that of standard antioxidant. Radical scavenging capacity of extract related to their concentration of total phenolic content (TPC). Many polyphenols contribute significantly to the antioxidant activity and act as a high effective free radical scavengers. These are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals **[**xxxxvi**].**

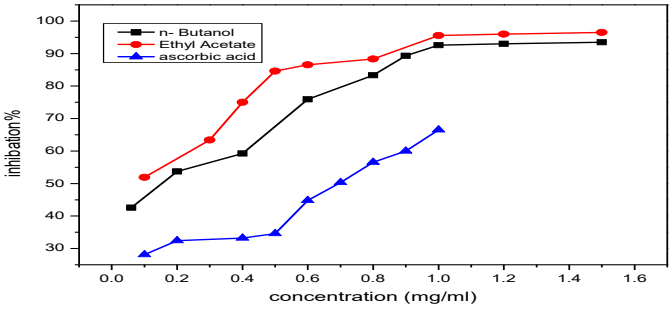

Figure 11: The DPPH radical scavenging activity of *M. Ciliata* extracts and the ascorbic acid.

3.3.1.b. Free superoxide anion radical ($O_2^{\cdot-}$) radical-scavenging ability

The following procedure was followed for the measurement of the antioxidant capacity:
It was obtained cyclic voltammograms of a solution of (DMF + 0.1M TNBHFP) recorded at a scan rate of 100 mV/s on GC at 28°C (A) saturated with commercial oxygen and are shown in Figure 12, and the same way this has been done cyclic voltammograms of extracts and the standard so that the sample solution is gradually added with different sizes from 0.1 to 1 ml so that the concentration in the cell changes with each addition and we draw the cyclic voltamperemetry curves for each addition under the same previous conditions are shown in Figure 13, Then We calculate the percentage of inhibition.

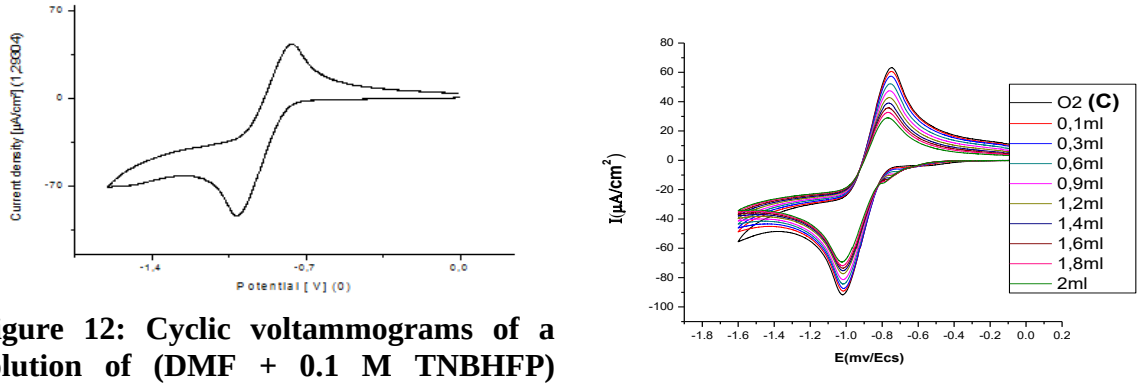

Figure 12: Cyclic voltammograms of a solution of (DMF + 0.1 M TNBHFP) recorded at a scan rate of 100 mV/s on GC at 28°C (A) saturated with commercial oxygen

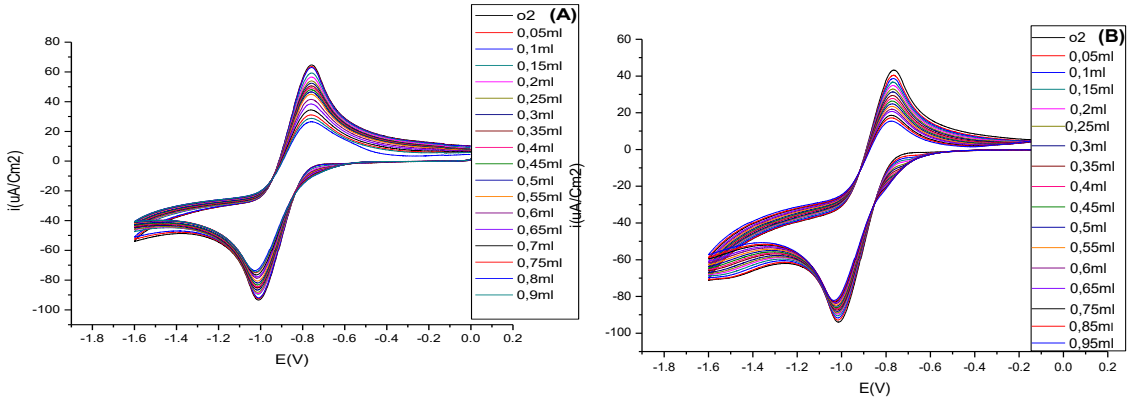

Figure 13: Cyclic voltammograms of $O_2^{\cdot-}$ in presence of (A) AcOEt extract ,(B) n-BuOH extract and (C) Quercetin at different concentrations in DMF +0.1M TNBHFP on GC as working electrode vs. Hg/Hg_2Cl as reference at 28°C with scan rate of 100 mV/s

The cyclic voltammograms shown in Figure 13 show that the addition of *M. Ciliata* extracts always causes a decrease in the current density of the anode peaks i_{pa} accompanied by a shift in the potential of the anode peaks to the more positive values. The antioxidant capacities obtained from decrease in anodic peak current, we also note that increasing the concentration leads to an increase in inhibition. It is well known that the addition of a flavonoid to the solution containing super oxide radical causes a decrease in the current (at least in one; cathodic or anodic) and a positive shift in the peak potential value, which is due to the scavenging activity of the added flavonoid and the extracts are rich in them **[**xxvii**].** The decrease in the current is directly proportional to the concentration of the radical and is a direct measure of the antioxidant activity **[**xxxxvii**]**.

3.3.2. The ferric reducing antioxidant power (FRAP) assay

The antioxidant activity of FRAP assay was calculated as ascorbic acid equivalent mg/g using the expression obtained from the calibration curve Figure 5, where the results were measured three times to test the reproducibility of the assay and the results obtained are shown in Table 3. EtOAc extract gave the best effective compared to ***n*-BuOH** extract, and that is because the flavonoids and phenolic acids are present in the medicinal plant exhibit strong antioxidant activity which is depending on their potential to form the complex with metal atoms, particularly iron and copper **[**xxxxviii**]**. This method is based on the principle of increase in the absorbance of the reaction mixtures, the absorbance increases the antioxidant activity increases. The antioxidant compound present in the samples forms a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm by UV-Spectrophotometer **[**xxxxix**]**.

3.3.3. Total antioxidant capacity (Molybdate ion reduction assay)

The total antioxidant capacity of studied *M. Ciliata* extracts was determined by molybdate ion reduction method and was calculated as ascorbic acid equivalent mg/g using the expression obtained from the calibration curve Figure 6 and the obtained results are shown in Table 3. The highest reducing power was found in the EtOAc extract. The high antioxidant activities of this plant extracts be due to their flavonoid and phenolic contents **[**xxxxx**].**

3.3.4. ABTS radical scavenging activity

ABTS assay was the most popular spectrophotometric method because they are simple, rapid, sensitive and reproducible. Its reaction involves an electron-transfer process. Bleaching of ABTS cation has been extensively used to evaluate the antioxidant capacity **[**xxxxvi**].** The extracts from *M. Ciliata* were fast and effective scavengers of $ABTS^{\cdot+}$ radical Figure 14 and the obtained results are shown in Table 3.

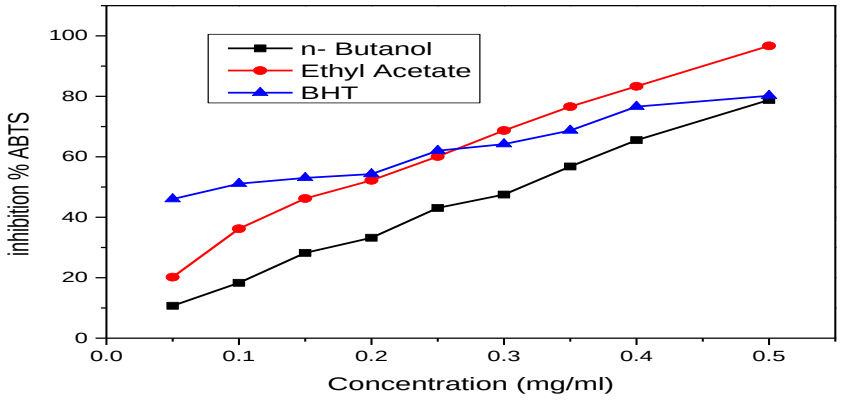

Figure 14: ABTS radical scavenging activities of extracts of *M.Ciliata*

This activity is comparable with that of BHT, the standard antioxidant used in this study. The percentage inhibition was 78.8%, 96.7% and 80.2% in n-butanol, ethyl acetate and BHT respectively at 0.5 mg/ml, the highest concentrations tested. Higher concentrations of the extracts were more effective in quenching free radicals in the system. EtOAc extract gave the best effective compared to *n*-BuOH extract. It turns out that there is a relationship between $ABTS^{\cdot+}$ radical scavenging activity for extracts and Total phenolic, flavonoids content and confirms this compounds have contributes directly to the antioxidant effects of these extracts.

4. CONCLUSION

Antioxidant capacity of *M. Ciliata* extracts was performed using many methods in parallel because different methods could give different results. The ethyl acetate extract exhibited the highest activity in the all assays followed by *n*-butanol extract. This also proves that polyphenolic compounds and antioxidant activities are closely linked. There was no previous study regarding antioxidant activity of the different polarity extracts (ethyl acetate and *n*-butanol) from *M. ciliata*.

No literature has been found regarding the total polyphenolic content and antioxidant capacity of different *M. Ciliata* extracts. The results obtained herein are in agreement to a certain degree with the traditional uses of *M. Ciliata* as a valuable source for antioxidant drugs.

5. ACKNOWLEDGEMENTS

The authors would like to thank to Algerian Ministry of Higher Education and Scientific Research for their support and providing the necessary facilities to carry out this research.

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Received on October 7, 2021.