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POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF AN ENDEMIC PLANT OF ALGERIAN SAHARA: *ANVILLEA RADIATA*

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Abstract

This study was carried out to assess the main secondary metabolites contents of *Anvillea radiata* plant using four solvents of increasing polarities (hexane, dichloromethane, methanol, and water), and three complementary antioxidant tests: total antioxidant capacity assay, DPPH free radical-scavenging ability, and ferric reducing antioxidant power (FRAP) assay. Methanol and Aqueous extracts recorded the highest extract yields (20.49±0.26 % and 11.58±0.23% respectively). Dichloromethane extract showed the highest rate of total phenols, flavonoids, and tannin contents (114.45±0.02 mg GAE/g d.w., 245.21±0.07 mg CE/g d.w., and 101.765±0.014 mg CE/g d.w respectively). Dichloromethane and Methanol extracts recorded a significantly high rate in total antioxidant activity (14.41±0.009 and 9.55±0.0023 mg GAE/g d.w. respectively) and exhibited a significant ability to scavenge DPPH radical (IC50 values = 0.9±0.026 and 1.75±0.051 mg/mL respectively) and a significant iron reducing power (EC50 = 0.98±0.034 and 1.31±0.043 mg/ml

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respectively). These results prove that *A. radiata* could indeed be a potential source of natural antioxidant and could be of considerable interest for the development of new medicines based on local plants to enhance the natural resources of the national heritage.

Introduction

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules and can damage cell structural molecules such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions (BIRBEN et al. 2012). In healthy aerobes organisms, the production of ROS is approximately balanced with antioxidant defense systems (HALLIWELL 2007). The shift in the balance between oxidants and antioxidants in favor of oxidants is termed "oxidative stress" (BIRBEN et al. 2012). Oxidative stress is implicated in the progression of several neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS) (BARNHAM et al. 2004). Growing evidence indicates that chronic and acute overproduction of ROS under pathophysiologic conditions is integral in the development of metabolic disorders such as diabetes and cardiovascular diseases (MADAMANCHI et al. 2005). It is also known that ROS can induce instability of the cell membrane (MORA et al. 1990), destruction of DNA structures (TAKABE et al. 2001), and induction of mutations (SASTRE et al. 2000), carcinogenic effects (KAWANISHI et al. 2001), and infertility (SHEWEITA et al. 2005). Many synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate have been widely used in different food products. However, because of the potential health hazards, their use as food additives is under strict regulation in many countries (WANG et al. 2009). Currently, many studies are devoted to exploring and utilizing natural antioxidants to remove excessive free radicals in the human body, thus preventing or treating diseases (LI et al. 2014). The Algerian flora is full of several plant species that offer real pharmacological properties, which is still little or not studied (BENTABET et al. 2014). This diverse flora is a natural reservoir of bioactive molecules (EL GUICHE et al. 2015). Total and perfect control of the different properties of these plants, which involves determining all of the physicochemical groups capable of producing one or more pharmacological effects, is today a goal that occupies an order first place (ABDELWAHED et al. 2007). *Anvillea radiata,* a wild plant included in the Asteraceae family is growing predominantly

in the steppes of North Africa (Algeria and Morocco). *A. radiata* is a small woody shrub, densely branched, 20–50 cm high. The leaves are green-gray, small, and roughly triangular, with a large petiole and strongly toothed limb. The big solitary capitules have a diameter of 3–5 cm, with long ligules. The flowers are all yellow-orange, the outside one is 25 mm lenth. It is usually flowering in spring but can flower throughout the year. It is widely used in traditional medicine for the treatment of dysentery, gastric-intestinal disorders, and chest cold and has been reported to have hypoglycemic activity as well as antifungal activity (BOUKHRIS et al. 2016). This study aims to enhance the local flora by discovering new compounds or active principles with therapeutic interests.

Materials and Methods

Plant Sampling

Phytochemical study and evaluation of antioxidant activity required plant material represented by an endemic plant of Algerian Sahara named *Anvillea radiata* or *"Nugd"* as a vernacular name (BENKHNIGUE et al. 2016), collected in May 2019 in the region of Tamanrasset in Algeria. The plant used was identified by Dr. Bekkouche Assia, a botanist at Salhi Ahmed university center of Naama in Algeria. The aerial part (Figure 1) of the dried plant was ground and used for the preparation of various extracts.

Fig. 1. The dry appearance of the aerial part of *Anvillea radiata*

Chemicals and Reagents

2-(3,4 Dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (Catechin; C), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 3,4,5-trihydroxybenzoic acid (Gallic acid; GA), 3-methoxy-4-hydroxybenzaldehyde (Vanillin), 5-(1,2-dihydroxyéthyl)-3,4-dihydroxyfuran-2-one (ascorbic acid), aluminum chloride ($AlCl_3$), ammonium molybdate ($(NH_4)_6Mo_7O_{24}$), Folin-Ciocalteu phenol reagent, hydrochloric acid (HCl), iron chloride ($FeCl_3$), potassium ferricyanide solution $K_3Fe(CN)_6$, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), and sulfuric acid (H_2SO_4). All chemicals used were obtained from either Sigma-Aldrich or Merck.

Preparation of Plant Extracts

The aerial part of *Anvillea radiata* (10 g) was weighed into a Soxhlet extractor thimble and placed in the extraction apparatus. Four solvents of increasing polarities (Hexane, Dichloromethane, Methanol, and Water) were measured into a 250 mL conical flask depending on the feed-to--solvent ratio (1:10 g/mL). A heating mantle was used to reflux the mixture for an extraction time of 6 h. After the extraction time has been reached, the extract solution was allowed to cool at room temperature. Then, filtered through a cone of filter paper (Whatman no 1), concentrated to dryness using a rotary evaporator, and stored at 4°C until use (ALARA et al. 2018). The extraction yield was calculated using the following formula:

$$\text{yield of extract [\%]} = \left(\frac{\text{weight of extracts from plant sample}}{\text{weight of dried plant sample}}\right) \cdot 100\%$$

Total Phenolic Content (TPC)

Total phenolic contents were assayed using Folin-Ciocalteu reagent, following the method described by SERAIRI-BEJI et al. (2018). An aliquot of diluted sample fraction was added to 0.5 mL distilled water and 0.125 mL Folin-Ciocalteu reagent. The mixture was shaken and incubated for 6 min before adding 1.25 mL Na_2CO_3 (7%). The solution was then adjusted with distilled water to a final volume of 3 mL and mixed thoroughly. After incubation in the dark, the absorbance was read at 760 nm versus a prepared blank. TPC was expressed as milligrams Gallic acid equivalents per gram dry weight (mg GAE/g d.w.) through the calibration curve with Gallic acid. The calibration curve range was 0–100 μg /mL (R^2 = 0.97). All samples were analyzed in three replications.

Total Flavonoid Content (TFC)

The total flavonoid content of the extracts has been determined using the colorimetric method as described by KIM et al. (2003). 100 µL of the extract was mixed with 0.4 mL of distilled water following with 0.03 mL of sodium nitrite solution 5% $NaNO_2$. After 5 min, 0.02 mL of $AlCl_3$ solution to 10% has been added. 0.2 mL of 1 M Na_2CO_3 solution and 0.25 mL of distilled water after 5 min. The whole is agitated using a vortex and absorbance was measured at 510 nm. TFC was expressed as mg catechin equivalent per gram dry weight (mg CE/g d.w.), through the calibration curve of catechin 0–500 µg/mL range (R^2 = 0.99). Samples were analyzed in triplicate.

Total Condensed Tannin Content (TCT)

Total condensed tannin was measured according to the method described by SERAIRI-BEJI et al. (2018). 200 µL of properly diluted sample was added to (3 mL) of (4%) vanillin solution in methanol, and 1.5 mL of concentrated hydrochloric acid (HCl) was added. After an incubation period of 15 min, the absorption was measured at 500 nm against methanol as a blank. The amount of TCT was expressed as milligrams catechin equivalent per gram dry weight [mg CE/g d.w.]. The calibration curve range of catechin was established between 0 and 400 mg/mL. Samples were analyzed in triplicate.

Total Antioxidant Capacity (TAC)

TAC was evaluated through the method described by SANCHEZ-MORENO et al. (1998). An aliquot (100 µL) of diluted extract fraction was combined with 1 mL reagent solution (0.3 N sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Methanol was used instead of a sample for blank. Mixtures were incubated in a boiling water bath for 90 min then cooled to room temperature. Their absorbance was measured at 695 nm against a blank. Antioxidant capacity was expressed as mg Gallic acid equivalent per gram dry weight (mg GAE/g d.w.). All samples were analyzed in triplicate.

DPPH Radical-Scavenging Activity

DPPH radical-scavenging activity was carried out following the method of SANCHEZ-MORENO et al. (1998). Rapidly 50 µL of each extracts at different concentrations (from 0.078 to 5 mg/mL) were added to 1.95 mL

of the methanolic solution of DPPH (0.025 g/L). At the same time, a negative control is prepared by mixing 50 µL of methanol with 1.95 mL of the methanolic solution of DPPH. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 515 nm. The scavenging activity was expressed as IC50 [mg/mL], the dose required to cause a 50% DPPH inhibition. A lower IC50 value corresponds to a higher antioxidant activity of plant extract. The percentage of radical-scavenging is calculated according to the following equation:

$$I\%=(A1 - A2/A1 \cdot 100$$

where:
A1 – absorbance of the control (DPPH solution without extract).
A2 – absorbance in the presence of extract.

Ferric Reducing Antioxidant Power Assay (FRAP)

The Ferric reducing antioxidant power assay is determined according to the method described by WU et al. (2014). One milliliter of the extract at different concentrations (from 0.078 to 5 mg/ml) was mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of a potassium ferricyanide solution $K_3Fe\ (CN)_6$ at 1%. The mixture is incubated in a water bath at 50°C for 20 min. thereafter, 2.5 mL of trichloroacetic acid at 10% is added to stop the reaction and the tubes are centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of supernatant is combined with 2.5 mL of water distilled and 0.5 mL of an aqueous 0.1% $FeCl_3$ solution. The absorbance of the reaction medium is read at 700 nm against a similarly prepared blank, replacing the extract with distilled water which allows calibrating the device (UV-VIS spectrophotometer). The positive control is represented by a solution of ascorbic acid and the absorbance was measured under the same conditions as the samples. An increase in absorbance corresponds to an increase in the reducing power of extracts tested.

Statistical Analysis

All assays were carried out in triplicate (n = 3) and their results were expressed as mean ± standard error of the mean and analyzed by SigmaPlot for Windows version 11.0. A comparison between groups was made using the Bonferroni test. Columns not sharing a common letter (a–c) differ significantly at $p < 0.05$ (Bonferroni test).

Results

Extraction Yield, Total Phenol, Flavonoid, and Condensed Tannin Contents

The extraction of the aerial part of *Anvillea radiata* using four solvents of increasing polarity allows us to calculate the extraction yields expressed in percentages relative to the initial dry weight. As shown in Figure 2,

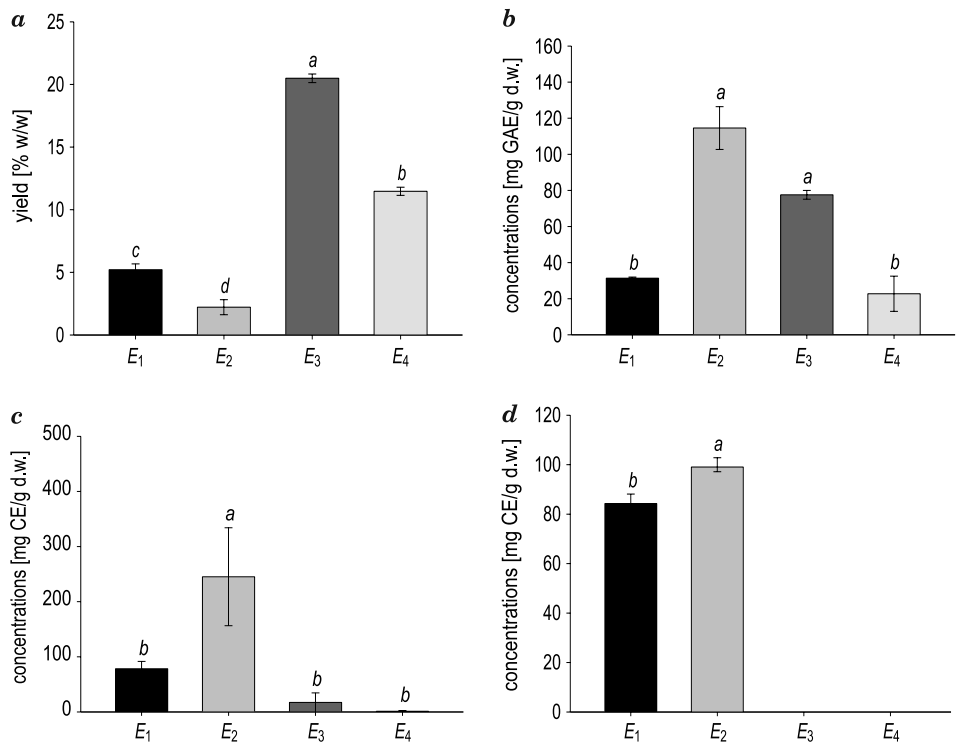

Fig. 2. Different extracts from *Anvillea radiata* (E_1 – hexane, E_2 – dichloromethane, E_3 – methanol, and E_4 – water extracts): *a* – extraction yield; *b* – total phenolic content; *c* – total flavonoid content; *d* – total condensed tannin. Data are expressed as means ± SE ($n = 3$). Comparison between groups was made using the Bonferroni test. Columns not sharing a common letter (*a*–*d*) differ significantly at $p < 0.05$ (Bonferroni test)

the highest yield is obtained in the methanol extract followed by the aqueous extract (20.49±0.26 % and 11.58±0.23 % respectively). Total phenol content was determined as Gallic acid equivalent in milligram per gram dry weight (mg GAE/mg d.w.). While total flavonoid and total condensed tannin were calculated as catechin equivalent in milligram per gram dry weight (mg CE/g d.w.). The total phenol contents of the dichloromethane

and methanol extracts from *Anvillea radiata*'s aerial part were significantly higher than the other extracts and determined to be 114.45±0.025 and 77.56±0.005 mg GAE/mg d.w. However, only the dichloromethane extract shows a significantly high level of flavonoids (245.21±0.074 mg CE/g d.w.) compared to the other extracts. While the highest level of condensed tannin is recorded for the dichloromethane extract (101.76±0.014 mg CE/g d.w.) followed by the hexane extract (86.76±0.003 mg CE/g d.w.).

Antioxidant Activities

Three complimentary tests were used in this study to assess the antioxidant activity of different extracts from *Anvillea radiata* aerial part: Total antioxidant capacity, DPPH free radical-scavenging activity, and reducing power assays. The total antioxidant activity was expressed as the number of Gallic acid equivalent. The study revealed that the dichloromethane extract recorded a significantly high rate in total antioxidant activity (14.41±0.009 mg GAE/g d.w.) compared to the other extracts. Results shows that both the dichloromethane and methanol extracts exhibited a significant ability to quench DPPH radical ($IC_{50\%}$ values = 0.9±0.026 mg/mL and 1.75±0.051 mg/mL respectively) and a significant iron reducing power (EC50 = 0.98±0.034 and 1.31 ± 0.043 mg/mL respectively) compared to the other extracts but that remain significantly lower than that of ascorbic acid ($EC_{50\%}$= 0.005 ± 0.003 mg/ml) (Figure 3 and Table 1).

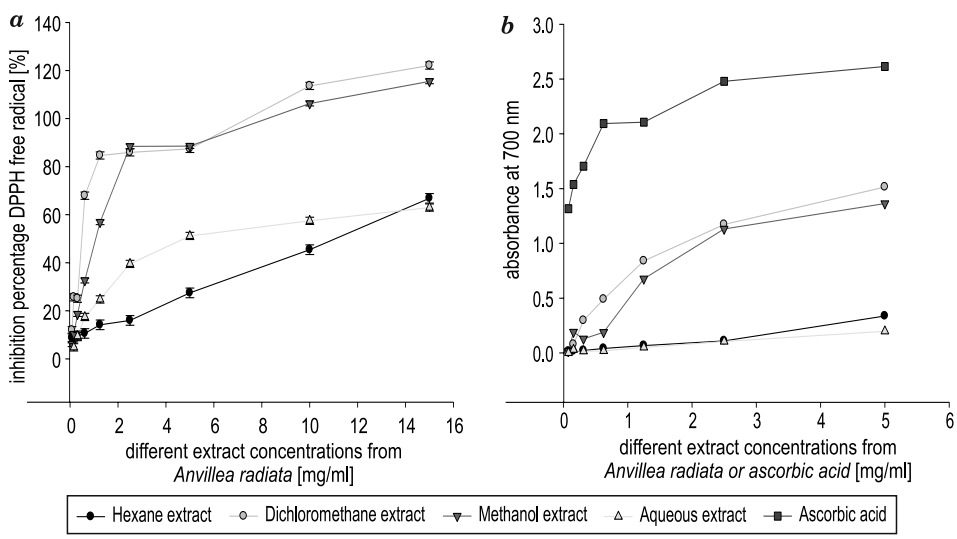

Fig. 3. Different extract concentrations from: *a* – *Anvillea radiata* – DPPH radical-scavenging activity; *b* – *Anvillea radiata* compared to ascorbic acid – ferric reducing antioxidant power assay (FRAP). Data are expressed as means ± SE (n = 3)

Table 1
Total antioxidant activity, DPPH radical-scavenging activity and Ferric reducing antioxidant power assay of the different extracts from *Anvillea radiata*

Extracts	TAC [mg GAE/g d.w.]	IC_{50} value of DPPH [mg/ml]	EC_{50} value of FRAP assay [mg/ml]
E_1	9.55 ± 0.002^b	10.7 ± 0.038^b	7.89 ± 0.060^c
E_2	14.41 ± 0.009^a	0.90 ± 0.028^a	0.98 ± 0.034^b
E_3	6.69 ± 0.0012^c	1.75 ± 0.019^a	1.31 ± 0.043^b
E_4	7.65 ± 0.0010^b	4.23 ± 0.034^b	12.82 ± 0.080^c
Ascorbic acid	–	–	0.005 ± 0.003^a

Explanation: E_1 – hexane; E_2 – dichloromethane; E_3 – methanol; E_4 – water extracts. Data are expressed as means±SE (n = 3). Comparison between groups was made using the Bonferroni test. Column not sharing a common letter (a–d) differ significantly at $p < 0.05$ (Bonferroni test)

Discussion

Nowadays, there is increasing attention to the health benefits of plant phenolic compounds due to their antioxidant activities (SERAIRI-BEJI et al. 2018). This study was conducted to characterize the phenolic profile and antioxidant potential of the extracts obtained from *Anvillea radiata* using four solvents of increasing polarities (hexane, dichloromethane, methanol, and water). Our results show that the highest levels of total phenols and total flavonoids from *A. radiata* were recorded for the dichloromethane and methanol extracts, whereas the most important values of condensed tannin contents were recorded for the dichloromethane and hexane extract. Nevertheless, our results remain higher than the results found by EL GUICHE et al. (2015), who indicate that the methanol extract of *A. radiata* has a total phenols content of 11.54 µg GAE/mg DM. and a total flavonoids content of 30.28 µg CE/mg DM. In the study conducted by HEBI and EDDOUKS (2018) concerning the preliminary phytochemical screening of *A. radiata*, several compounds of chemicals have been found such as polyphenols, flavonoids, tannins, mucilage, sesquiterpenes, terpenoids, and carbohydrates. The total phenolic and flavonoid contents vary according to the plant organ used, the species analyzed, and the choice of solvent (XU and CHANG 2007). SAHREEN et al. (2017), report similar findings and indicate that the methanol extract had illustrated the highest total content of phenolic, whereas the content obtained with ethyl acetate was much lower, and justify that this can be due to the formation of complexes by a part of phenolic compounds with carbohydrates and proteins, which are more extractable in methanol than in other solvents. Our results do not deviate

from those of KANDOULI et al. (2017), who found the same results regarding the levels of total phenols and flavonoids using methanol as solvent (65.8±1.8 mg GAEC/g and 48.4±0.9 mg RUE/g respectively). An imbalance in the oxidant/antioxidant status is mostly accompanied by tissue injury and human disease, creating oxidative stress, which must be amenable to therapeutic intervention with appropriate antioxidants, provided that they can reach the site of damage and are effective in decreasing oxidative damage levels (HALLIWELL 2001). Therefore, because of the potential health hazards of many synthetic antioxidants widely used in different food products, which involve toxic side effects, their use is under strict regulation in many countries (WANG et al. 2009). That is why; actually there is a growing interest in the substitution of synthetic antioxidants used in food preservation with natural ones (SERAIRI-BEJI et al. 2018). Indeed, polyphenols are natural compounds widely distributed in the plant kingdom which have increasing importance in particular thanks to their beneficial effects on health (KOECHLIN-RAMONATXO 2006). According to ADEBIYI et al. (2017), the antioxidant activity of polyphenols is largely due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers, and as well as potential metal chelators. In this study, three complementary tests were used to assess the antioxidant activity of *Anvillea radiata* (Total antioxidant capacity, DPPH free radical-scavenging activity, and ferric reducing antioxidant power assay). The results showed that both dichloromethane and methanol extracts from *Anvillea radiata* showed strong antioxidant activity. These same extracts had the highest values in polyphenol and flavonoid contents, which confirm the correlation that exists between the content of phenolic compounds of an extract and its antioxidant activity. About that, KSOURI et al. (2009) has pointed out that there is a real positive correlation between antioxidant potential and phenolic content. Other works have shown that many flavonoids and related polyphenols significantly contribute to the total antioxidant activity of many fruits such as red grape (NEGRO et al. 2003).

Conclusion

The results of the present investigation indicate that the Dichloromethane and Methanol extracts of *Anvillea radiata* have the highest levels of the secondary metabolites tested and exhibit an important antioxidant activity in vitro, which confirms the correlation between the content of phenolic compounds and antioxidant activity. This finding provided

a strong evidence that the studied plant might indeed be a potential sources of natural antioxidant which could be of considerable interest to the development of new drugs based on local plants to enhance the natural resources of the national heritage.

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