

BIOACTIVITY OF *Anvillea radiata* COSS & DUR. COLLECTED FROM THE SOUTHEAST OF MOROCCO

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Abstract

The in vitro antimicrobial and antioxidant activities of aqueous, methanolic and ethyl acetate extracts of *Anvillea radiata* (Asteraceae) were investigated. Antibacterial activity was tested against six pathogenic strains viz. *Staphylococcus aureus* (ATCC 29213), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Salmonella abony* (NCTC 6017), *Escherichia coli* (ATCC 25922), *Escherichia coli* (ATCC 8739) and by using Disc diffusion method and Minimum Inhibitory Concentrations (MIC). Total antioxidant capacities were assessed by DPPH (1.1 diphenyl-2-picrylhydrazyl) radical scavenging activity, ferric reducing power (FRAP) and ABTS (2.2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging activity. Total phenolic contents were measured by Folin-Ciocalteu assay. Among the extracts tested, methanolic extract showed promising antibacterial activity against bacteria and reasonable antioxidant properties, and they can therefore be potentially used as a natural additive in food, cosmetic and pharmaceutical industries.

Keywords: *Anvillea radiata*, Antioxidant activity, Antibacterial activity. Phenolic content

Introduction

People have explored the nature especially plants in search of new drugs (Verpoorte, 1999, Dabai et al., 2012). Medicinal plants are a rich source of antimicrobial agents and are widely used in human therapy, veterinary, agriculture, scientific research and countless other fields (ukuri et al., 2009, Hussain et al., 2012).

The therapeutic benefits of the medicinal plants are often attributed to their antioxidant properties in relation to their large content on antioxidant compounds such as vitamin C, Vitamin E, polyphenol and flavonoids (Hertog et al., 1993, Zhang et al., 2001, Rice-Evans, 2004, Dixon et al., 2005).

Although many plant species have been tested for antimicrobial properties, a large majority have not been adequately assessed (Balandrin et al., 1985). In this context, *Anvillea radiata*, a Moroccan-Algerian endemic belonging to the Asteraceae family. This plant is widely used in Moroccan and Algerian traditional medicine for the treatment of several diseases: gastroenteritis, spasms, and colic, arthritis and rheumatoid (Bellakhdar, 1997, El Rhaffari and Zaid, 2002) of leucorrhoea, the hepatitis, diabetes and stomach pain (El Rhaffari and Zaid, 2002, Djellouli et al., 2013, Douira et al., 2013), indigestion and lung diseases (Djellouli et al., 2013). The main objective of this work was to determine the antioxidant and antimicrobial capacities of aqueous, methanolic and ethyl acetate fractions of *Anvillea radiata* harvested from the Tafilalet region in the Southeast of Morocco.

Materials and methods

Plant materials: At flowering stage, fresh aerial parts of *Anvillea radiata* were collected from the area of Tafilalet (Southeast of Morocco) (latitude: 32.204, longitude:-4.383, altitude: 1239 meters). The voucher specimen was deposited in the herbarium of the Faculty of Sciences & Technology, Errachidia, Morocco.

Preparation of Plant Extracts

Organic extracts: Air dried aerial parts of *A. radiata* (1085.2 g) were extracted too times with 2 L of MeOH. The MeOH extract was concentrated to dryness (89.6 g); the residue was dissolved in water (200 ml). The aqueous solution was partitioned successively three times with hexane (3×100 ml) giving 3.1g of hexane fraction, ethyl acetate (3×100 ml) giving 16.6 g of ethyl acetate fraction, and three times with n-BuOH (3×100 ml) giving 2 g of n-BuOH fraction.

Aqueous extract: The dried ground aerial parts (100g) of *A. radiata* were extracted with boiling distilled water (1L) for 24 hours. The recovered extract was filtered and concentrated using a rotary evaporator (Buchi

Labortechnik AG, Switzerland) under vacuum to obtain a dried powder. 27,1g of aqueous extract were obtained with 27.1% extraction yield.

Measurement of total phenolic compounds: The total phenolic contents in various extracts were determined according to (Singleton and Rossi, 1965) method. Briefly, 30 μL of the Sample (0.005 g mL^{-1}) was added to 250 μL Folin-Ciocalteu reagent, mixed for 3 min then 500 μL sodium carbonate solution (0.1 g mL^{-1}) was added. The volume was adjusted to 5 ml with distilled water and mixed. The mixture was left for 30 min at room temperature in a dark place and the absorbance was measured at 725 nm. The calibration curve was prepared using a concentration from (0 to 0.03 g l^{-1}) of caffeic acid. The total phenolic compounds were expressed as mg of caffeic acid equivalent per 1 g of extract.

Antioxidant activity

The antioxidant activity was assessed by ABTS radical cations (ABTS^+), 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging and the ferric reducing activity power FRAP.

Ferric reducing antioxidant power assay: The ferric reducing activity was estimated based on the method of (Benzie and Strain, 1999). The FRAP reagent was prepared by mixing 50 mL of acetate buffer (0.3M) at pH 3.6, 5 mL tripydyltriazine (TPTZ) solution 10 mM prepared in Hcl (40 mM) and 5mL of Ferric chloride solution (FeCl_3) (20 mM). 2 mL of the freshly prepared FRAP reagent was added to 10 μL of various extracts (5 mg mL^{-1}). Then the absorbance was measured at 593 nm against the blank after 10 minutes at room temperature. The standard curve was constructed using Trolox with concentration in the range of 0 to 3.08 mg l^{-1} . The result was expressed as mg of Trolox equivalent per 1 g of extract.

DPPH radical scavenging activity: Scavenging Radical activity of the various extract against stable DPPH was assessed as described by (Blois, 1958) method with slight modification. The reaction mixture contained 100 μL of each extract at concentration ranging from 5 to 0.013 g L^{-1} and 1.9 mL of methanolic DPPH (0.3 mM). The mixture was incubated at room temperature for 20 min and the absorbance was determined at 517 nm (Jenway UV/Vis 6000). The IC_{50} (concentration providing 50% inhibition) values were calculated from the plotted graph of scavenging activity against the concentrations of the samples. The capability to scavenge the DPPH radical was calculated using the following equation (Barros et al., 2007):

$$\text{DPPH-scavenging effect (\%)} = 100 * (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$$

The BHT was used as positives control.

ABTS radical scavenging assay: The ABTS radical scavenging was measured using the method of (Re et al., 1999). The ABTS radical cations

(ABTS⁺) were produced by reacting aqueous solution of ABTS (7 mM) with aqueous solution of potassium persulphate (2.45 mM). The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use, then diluted with distilled water to obtain an absorbance of 0.700 ± 0.005 at 734 nm. 30 μL of the sample (10 mg mL^{-1} for ME, 5 mg mL^{-1} for AE and AEE) added to 3 mL of the ABTS radical solution were allowed at room temperature for 6 min and the absorbance at 734 nm was recorded immediately. A standard curve was obtained by using aqueous solution of Ascorbic acid with concentration ranged (0 to 4.45 mg L^{-1}). Total antioxidants were expressed as mg of ascorbic acid equivalents per 1 g of extract. The BHT was used as positives control.

Antibacterial activity

Microorganisms used: The antimicrobial activity was evaluated by paper disc diffusion and micro dilution methods against sex selected species associated with various forms of diseases, three Gram-positive: *Staphylococcus aureus* (ATCC 29213), *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633), and three Gram-negative: *Escherichia coli* (ATCC 25922), *Escherichia coli* (ATCC 8739) and *Salmonella abony* (NCTC 6017). Microorganisms were obtained from the culture collection of the Institute of Hygiene (Rabat).

Disc-diffusion test: The qualitative antimicrobial essay of the extracts of *Anvillea radiata* was carried out by the disc diffusion method (NCCLS, 1999). It was performed using culture growth at 37°C for 18h and adjusted to approximately 10^8 colony forming unit per milliliter (CFU/ml). The culture medium used for the bacteria was Mueller Hinton Agar (MHA). Five hundred microliters of the inoculums were spread over plates containing MHA and a Whatman paper disc (6 mm) impregnated with $10 \mu\text{l}$ of the different concentration of extracts (0.1 mg/disc , 0.05 mg/disc) was placed on the surface of the media. The plates were left 30min at room temperature to allow the diffusion of extracts. They were incubated 24h at 37°C . After incubation period, the inhibition zone obtained around the disc was measured. Two controls were also included in the test, the first was involving the presence of microorganisms without test material and the second was standard antibiotic to control the sensitivity of the tested bacteria. The experiments were run in triplicate, and the developing inhibition zones were compared with those of reference discs.

Minimum Inhibitory Concentration (MIC): The antibacterial activity of the various plant extracts was assayed using a slight modification of the microdilution techniques described by Drummond and Waigh (Drummond and Waigh, 2000). An indicator solution was prepared by dissolving one tablet of resazurin dye in 40 ml of sterile water. An overnight

culture of a test bacterium in nutrient broth was diluted serially in 0.1% physiologic water to obtain 10^6 colony forming units/ml of broth culture. Extract solution was serially diluted in an appropriate solvent (water or dimethylsulphoxide) and placed in microtitre wells so that each well contained 100 μ l of a dilution.

The wells were labeled 1 – 10. An equal amount of broth culture (100 μ l) and indicator solution (100 μ l) were placed one after the other in each labeled well. Growth control solution comprised indicator solution and broth culture, while the sterile control consisted of indicator solution and sterile broth. The microtitre tray was incubated at 37°C for 6 hours. Blue coloured solution meant growth inhibition in test wells, while pink coloured solution indicated growth or absence of inhibition (Bitu et al., 2012).

Statistical analysis: The data obtained were analyzed by Student's-t-test. P values less than 0.05 were considered significantly different. Results are expressed as means \pm SD and all tests were carried out in an identical condition.

Results and discussion

Total phenolic content: The contents of total phenols measured by Folin Ciocalteu reagent in terms of caffeic acid equivalent are presented in Table 1. All extracts exhibited high polyphenol content, ranging from 216.31 to 93.66 mg CAE g^{-1} of extract. The Methanolic contained the highest amount of total phenols, followed by Ethyl Acetate extract. The aqueous extract contained the lowest amount with significant differences between all the extracts. This result concurred with those of Loganayaki and al., (2013) who reported that the total phenolic content was found to be higher in Methanolic extract of *Ceiba pentandra* (Loganayaki et al., 2013). The solubility of phenolics is governed by the chemical nature of the plant sample, as well as the polarity of the solvents used (Dai and Mumper, 2010).

Djeridane and al., (2010) showed that the extract of *Anvillea radiata* has a polyphenol content equal to 14.36 mg GAE g^{-1} dry matter (Djeridane et al., 2010).

Indeed, the phenolic contents of a plant depend on a number of intrinsic (genetic) and extrinsic (environmental, handling and storage) factors (Fratianni et al., 2007, Rapisarda et al., 1999).

Table 1. Total phenolic content in *Anvillea radiata* extracts

Extracts	Total phenolic*
Aqueous	93.66 \pm 2.85 ^a
Ethyl Acetate	127.54 \pm 1.79 ^b
Methanolic	216.31 \pm 6.14 ^c

*: Values expressed as mg CAE g^{-1} of extract (caffeic acid); For experiments \pm standard deviation. Mean values followed by different superscript in a column are significantly different (p<0.05)

Antioxidant potential

The potential antioxidant activity of aqueous and organic extracts from *Anvillea radiata* were determined by conducting three complementary tests, namely DPPH free radical scavenging, ferric reducing antioxidant power (FRAP) and Assay of ABTS radical scavenging activity. Total antioxidant activity of the plant extracts are recommended to carry out by using two or more methods (Politeo et al., 2007).

Based on this recommendation, three complementary test systems were used to evaluate the antioxidant properties of the various extracts of *Anvillea radiata*. In the three test systems, the different extracts exhibited antioxidant properties.

DPPH[•] radical scavenging activity: The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh et al., 2009a). DPPH is a stable nitrogen centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen - or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Brand-Williams et al., 1995). IC₅₀ for DPPH radical-scavenging activity value was 91.95±1.04 µg ml⁻¹ for ME. The IC₅₀ values for EAE, AE and BHA were 87.06±1.43, 265.52±18.02 and 72.23±1.98 µg ml⁻¹, respectively (Table 2). Djeridane and al., (2010) reported that the extract of *Anvillea radiata* have IC₅₀ equal value to 21.73±0.09 mg ml⁻¹ (Djeridane et al., 2010).

Table 2. Antioxidant capacity of *Anvillea radiata* extracts

Extracts	FRAP*	DPPH**	ABTS***
Aqueous	48.40±3.26 ^a	265.52±18.02 ^a	19.95±0.91 ^a
Ethyl Acetate	60.62±1.67 ^b	87.06±1.43 ^b	44.45±0.78 ^b
Methanolic	109.70±2.44 ^c	91.95±1.04 ^c	67.06±1.47 ^c
BHT	-	65.23±1.98 ^d	71.62±1.67 ^c

*: Values expressed as mg TE g⁻¹ of extract (Trolox); **: IC₅₀ en µg ml⁻¹; ***: Values expressed as mg AASE g⁻¹ of extract (Ascorbic acid).

Mean values followed by different superscript in a column are significantly different (p<0.05)

Ferric-reducing antioxidant power: The FRAP assay is an electron transfer-based test measuring the substance ability to reduce Fe³⁺ to Fe²⁺ (Krishnaiah et al., 2011). Results of FRAP assay were expressed as mg TE g⁻¹ of extract, and higher FRAP values were interpreted as higher antioxidant activity, as opposed to IC₅₀ DPPH. FRAP value was the lowest in aqueous extract (48.40±3.26 mg TE g⁻¹ of extract) and the highest in Methanolic extract (109.70±2.44 mg TE g⁻¹ of extract) (Table 2).

ABTS cation radical scavenging activity: ABTS assay is an excellent tool to determine the antioxidant activity of hydrogen-donating

antioxidants (scavenging aqueous phase radicals) and of chain breaking antioxidants (scavenging lipid peroxy radicals). The total antioxidant activities of different extracts are presented in Table 2. The total antioxidant activity of the sample extracts ranges from 19.95 to 67.06 mg AASE g⁻¹ of extract and the values are significantly (p<0.05) different. The Methanolic extract exhibited higher and comparable activity (67.06±1.47 mg AASE g⁻¹ of extract) on a par with the standard antioxidant BHT (71.62±1.67 mg AASE g⁻¹ of extract).

Antioxidant activity of *Anvillea radiata*, as shown by the three assays used, could be related to their different phenolic content and composition. In other words, antioxidant activity increased proportionally to the phenolic content. Phenols and polyphenolic compounds, such as flavonoids, are widely found in plants, and they have been shown to possess significant antioxidant activities (Ebrahimzadeh et al., 2009b).

Antibacterial activity: The results of susceptibility of the strains tested are indicated in table 3. Standard antibiotics also exhibited marked activity against Gram positive bacteria than Gram negative bacteria, with the exception of Penicillin which is inactive against both strains of *E. coli* and *S. aureus* ATCC 25923.

Table 3. Susceptibility of the strains tested

Microorganisms	Antibiotics					
	P ₁₀	C ₃₀	CN ₁₀	S ₁₀	AMC ₃₀	CIP ₅
<i>E. c</i> ATCC 25922	NA	23.33±0.57	21.67±0.57	12.33±0.57	10.33±0.57	23.67±1.11
<i>E. c</i> ATCC 8739	NA	26.33±0.57	21.67±0.57	12.00±0.00	17.00±1.00	12.33±0.57
<i>S. a</i> ATCC 25923	NA	17.00±1.00	23.67±1.11	21.67±0.57	34.33±1.15	30.33±0.43
<i>S. a</i> ATCC 6538	34.33±1.15	29.00±1.00	24.66±0.57	20.00±0.00	33.66±0.57	15.33±0.57
<i>S. ab</i> NCTC 6017	15.33±0.57	26.33±0.57	21.00±1.00	16.66±0.57	23.33±0.57	14.67±0.43
<i>B. s</i> ATCC 6633	34.66±0.57	35.33±1.15	30.66±0.57	21.33±0.57	29.66±1.15	21.67±0.57

P₁₀ : Penicillin ; C₃₀ : Chloramphenicol (30µg/disc) ; CN₁₀ : Gentamicin (10µg/disc) ; S₁₀ : Streptomycin (10µg/disc) ; AMC₃₀ : Amoxycillin/Calvulanic Acid (30µg/disc) ; CIP₅ : Ciprofloxacin (5µg/disc).

E. c : *Escherichia coli* ; *S. a* : *Staphylococcus aureus* ; *S. ab* : *Salmonella abony* ; *B. s* : *Bacillus subtilis*.

The results of antimicrobial activity of the aqueous, Methanolic and Ethyl acetate fraction of *Anvillea radiata* are indicated in table 4. Preliminary evaluation of antibacterial activity was carried out by disk diffusion method in solid medium. This method has shown that all extracts (aqueous and solvent extracts) in study demonstrated activity against species of bacteria tested, suggesting that the medicinal plants could be an important alternative in control of bacterial. This activity is mainly due to its bioactive compounds with an inhibitory effect on bacterial growth. The aerial part of *Anvillea radiata* contains various chemical compounds (Alkaloids, flavonoids, terpenoids, saponins, sterols, tannins, anthracenosides, emodols, anthraquinones) (Djellouli et al., 2013, Lakhdar et al., 2013).

S. abony NCTC 6017 was the most sensitive to the action of ME at 1mg/disc concentration, under an inhibition zone diameter of 13.75 ± 0.50 mm followed by *S. aureus* ATCC 6538 (12.25 ± 0.50 mm) and *B. subtilis* ATCC 6633 (12.00 ± 0.00 mm). Whereas EAE was more active on *E. coli* ATCC 8739 (12.50 ± 0.57 mm) and *B. subtilis* ATCC 6633 (13.00 ± 0.81).

The AE and the EAE showed no activity against Gram-negative bacteria *E. coli* ATCC 25922. Urzua and al. and Joshi and al., suggested that the outer membrane of gram-negative bacteria could act as a barrier against the active substances present in extracts of plants (Urzua et al., 1998, Joshi, 2013). This result concurred with those of Bammou and al., (2014) who reported that the crude aqueous extracts presents an antibacterial effect against some pathogens and multidrug-resistant strains (Bammou et al., 2014). In addition, the methanol extract of *Anvillea garcini* showed good activity against *E. coli* and *P. aeruginosa* (Javidnia et al., 2009).

B. El Hassany and al., (2004) reported that 9a-hydroxyparthenolide isolated in *Anvillea radiata*, at a concentration of 50 and 100 $\mu\text{g}/\text{disc}$, inhibited the growth of *Bacillus cereus* (IPL 58605), *Streptococcus C* (IPT 2-035), *Proteus vulgaris* (CIP 58605); *Enterococcus faecalis* (CIP 103214) and *Escherichia coli* (CIP 54127) (El Hassany et al., 2004).

Some advantages over the agar disk-diffusion method include the use of small quantities of extract, and the ability to distinguish between bacteriostatic and bactericidal effects besides the MIC determination. Also, the use of a colorimetric indicator eliminates the ambiguity associated with visual comparison (Langfield et al., 2004).

Table 4. Antibacterial activity of *Anvillea radiata* extracts against the bacterial strains based on disc diffusion method

Microorganisms	Extracts (mg/discs)					
	AE		ME		EAE	
	0.1	0.05	0.1	0.05	0.1	0.05
Gram-negative						
<i>E.c</i> ATCC 25922	NA	NA	10.25 ± 0.50	NA	NA	NA
<i>E.c</i> ATCC 8739	12.50 ± 0.57	10.75 ± 0.50	11.25 ± 0.50	09.75 ± 0.50	12.50 ± 0.57	10.25 ± 0.50
<i>S. ab</i> NCTC 6017	12.25 ± 0.50	11.75 ± 0.95	13.75 ± 0.50	12.50 ± 0.57	13.00 ± 0.57	12.57 ± 0.95
Gram-positive						
<i>S. a</i> ATCC 25923	10.25 ± 0.50	NA	11.00 ± 0.81	NA	11.75 ± 0.50	NA
<i>S. a</i> ATCC 6538	11.00 ± 0.00	10.25 ± 0.50	12.25 ± 0.50	7.25 ± 0.50	11.00 ± 0.50	10.57 ± 0.50
<i>B. s</i> ATCC 6633	12.25 ± 0.50	11.00 ± 0.00	12.00 ± 0.00	11.75 ± 0.50	13.00 ± 0.81	11.75 ± 0.50

E.c : *Escherichia coli* ; *S.a* : *Staphylococcus aureus* ; *S.ab* : *Salmonella abony* ; *B.s* : *Bacillus subtilis*.

AE: Aqueous extract; ME: Methanolic extract; EAE: Ethyl acetate extract; NA: Not active.

Based on the results of dilution method, Minimum inhibitory concentration values of *Anvillea radiata* extracts on different kinds of bacteria are summarized in Table 5. The aqueous extract showed MIC values ranging between 6.66 and 1.66 mg ml⁻¹. Methanolic extract presented MIC against strains bacterial tested (5-0.42 mg ml⁻¹), the ME extract who exhibited higher degree of anti-bacterial activity (0.42 mg ml⁻¹) against *S. abony* NCTC 6017.

Table 5. The MIC values of different extracts from *Anvillea radiata* against the microorganism

Microorganisms	MIC (mg ml ⁻¹)		
	AE	ME	EAE
Gram-negative bacteria			
<i>E.c</i> ATCC 25922	6.66	5.00	6.66
<i>E.c</i> ATCC 8739	3.33	5.00	1.66
<i>S. ab</i> NCTC 6017	1.66	0.42	0.83
Gram-negative bacteria			
<i>S. a</i> ATCC 6538	6.66	5.00	1.66
<i>S. a</i> ATCC 25923	3.33	3.33	1.66
<i>B. s</i> ATCC 6633	3.33	3.33	1.66

E.c : *Escherichia coli* ; *S.a* : *Staphylococcus aureus* ; *S.ab* : *Salmonella abony* ; *B.s* : *Bacillus subtilis*.

AE: Aqueous extract; ME: Methanolic extract; EAE: Ethyl acetate extract; NA: Not active.

Conclusion

This study has demonstrated a comparative account of antioxidant and antibacterial activities of various extracts of areal parts of *Anvillea radiata* collected from Southeast region in Morocco. Considering the results obtained, we can conclude that all extracts showed promising antimicrobial and antioxidant activities, among various extracts. Methanol was found as a better solvent for extraction of antioxidant and antibacterial substances. The study revealed that the methanolic extract contains a considerable quantity of phenolic compounds, more than what is in the other extracts. The antioxidant and antibacterial activities were mainly due to these phenolic compounds.

Thus, the aerial part of *Anvillea radiata* can be considered as a potent source of natural antioxidants and antibacterial agents and can be exploited for developing nutraceutical and pharmaceutical products.

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