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Chemical Composition, In-Vitro Anti-microbial and Antioxidant Activities of the Methanolic Extract of *Anvillea Radiata Asteraceae.*

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ABSTRACT

Anvillea radiata Coss. (Asteraceae) is a wild plant endemic of North Africa (steppes of Algeria and Morocco).It is widely used by local people for its medicinal properties. Phytochemical tests applied for *Anvillea radiata* showed the presence of several families of chemical compound like flavonoïds, saponins, steroids, fatty acids and tannins. The antioxidant activity was evaluated by the DPPH (1,1-diphenyl-2-picrilhydrazyl) and Ferric reducing antioxidant power (FRAP) assays. The methanolic extract of the plant showed significant DPPH radical scavenging activity comparable to butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA) and Ascorbic acid, with an IC_{50} value of 23.16 mg/l. The reducing power was also found to be concentration dependent. The antibacterial bioassay was studied by disc diffusion and minimum inhibitory concentration procedures against 5 bacteria (both gram positive and gram negative).The results revealed that the methanolic extract is effective mostly against (Streptocoque Staphylococcus aureus, pseudomonas aeriginosa, klebsiella pneumonie, with MIC between 75 µg/ml and 300 µg/ml. Our results indicate that this plant would be able to promise sources of natural products with potential antibacterial and antioxidant activities.

Keywords: antibacterial activity, antioxidant activity, *Anvillea radiata*, methanolic extract.

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INTRODUCTION

Because of their therapeutic properties, medicinal plants are one way of investigation the most interesting image of the discovery of new drugs from plants used in traditional medicine for the treatment of discharged diseases (Djellouli1 et al., 2013). Majority of these plants have been used in extensive application against human and animal pathogens. However, drug resistance of human pathogenic bacteria has been documented globally and thus natural product from plant origin is heavily dependent by man for the treatment of diseases (Oyedemi and Afolayan, 2011). nowday, antioxydant agents of plant source have attracted special interested because they can protect human body from diseases induced by free radicals with little or no side effects (Oyedemi and Afolayan, 2011).

Several synthetic drugs, such as butylated hydroxytoluene (BHT) and butylated hydroxyl anisole (BHA) are commonly used as antioxidants, but they have been reported to cause tissue toxicity, cell damage, inflammation and atherosclerosis in both animals and humans. Recent findings clearly show that the consumption of plant foods and natural antioxidant supplements may be used to protect the body against various diseases, including cancer, cardiovascular and neurodegenerative diseases (Sen et al., 2013; Boulekbache et al., 2012).

Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Balandrin et all., 1985; Mebarki et all., 2013). In this context, Anvillea radiata Coss. (Asteraceae) (Quezel, 1963; Ozenda, 1958) Vernacular name (*Nogde*), that grows in the two Maghreb countries , Algeria and Morocco (Dendougui, 2006). It is widely used in traditional medicine for the treatment of dysentery, gastric-intestinal disorders, chest cold and has been agents and they are widely used in the human therapy (Mebarki et al., 2013), reported to have antioxidant and antibacterial activities.

MATERIALS AND METHODS

Chemicals

Chemicals were purchased from Sigma-Aldrich (Steinheim, Germany and Merck (Germany), Biochem, Chemopharma (Montreal, Quebec), Applichem Panreac (Barcelona, SPAIN), Sigma (USA), Aldrich (Milwaukee, USA), All chemicals and solvents used were of analytical grade.

Plant Material

At flowering stage, Fresh parts (leaves and flowers) of *Anvillea radiata* were collected at May 2011 from the area of Dhait Ben Saleh, El Djelfa (Algeria) .The harvested parts were shade dried. After drying, the plant materials were ground well in a mortar and transferred into airtight containers for future use.

Phytochemical screening

Phytochemical tests were performed on different extracts prepared from the dried plant materials and ground, using four solvents of different polarities: water, ethanol, diethyl ether and petroleum ether. Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by various authors (Trease and Evans, 1989; Bruneton, 1999)

Preparation of plant extract

The powdered plant materials (250 g) were extracted with petroleum ether twice then three times with 70% MeOH (1.5 l) at room temperature 48h. The methanol extracts were combined and concentrate under reduced pressure on a rotary evaporator to dryness (Dendougui et al., 2006) and then lyophilized. The extract was stored at 4°C until tested (Ahmad et al., 2005).

Determination of phenolic composition

The amount of total phenolic in the extract was determined using the Folin–Ciocalteu reagent and Gallic acid as standard as described by (Obiang-Obounou, 2013). The total phenolic content (TPC) was

expressed as gallic acid equivalents (GAE) in mg per 100 g of dry weight. The concentration of phenolic compounds was calculated according to the following equation that was obtained from standard gallic acid graph.

Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) was determined according to the aluminum chloride colorimetric method described of (Wang et al., 2008; Benaissa et al., 2013). Total flavonoid content was expressed as mg Quercetin equivalents (QE) per 100 g of dry weight. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard Quercetin graph.

DPPH radical scavenging activity assay

Radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically by the slightly modified method of (Djeridane et al,2010), as described below. The solution of DPPH in methanol (250 µM) was prepared daily, before measurements. Various concentrations of 1 ml of sample solution diluted in Tris buffer solution (100 mM; pH = 7.4) were added to 1 ml of the DPPH radical solution. The mixture was then shaken vigorously and allowed to standart at room temperature in the dark for 30 min. The decrease in absorption was measured at 517 nm.

The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: IC_{50} amount of dry material, which causes 50% inhibition of DPPH radicals in reaction mixture.

Activity % = [(absorbance of control − absorbance of sample) / absorbance of control] × 100. (Arabshahi and Urooj, 2006).

The extract was compared with that of ascorbic acid, BHT and BHA.

Reducing power

The reducing power of the extract was determined according to the method of (Budrul Alam, 2012; Arabshahi and Urooj, 2007; Oyedemi and Afolayan, 2011) with some modifications. Methanolic extract, ascorbic acid, BHA and BHT were used at differing concentrations (0.01 to 0.1 mg/ml). One milliliter of either Methanolic extract, ascorbic acid, BHA or BHT was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide $[K_3Fe(CN)_6]$. The mixture was incubated at 50 °C for 20 min. A 2.5 ml of 10% TCA was added to the mixture, exactly 2.5 ml of the solution after vigorous shaking was mixed with distilled water (2.5 ml) and 0.5 ml of 1% $FeCl_3$. The absorbance was measured at 700 nm in a spectrophotometer. All determinations were carried out in triplicate.

Antibacterial bioassay (disc diffusion method)

The methanolic extract was tested against five microorganisms. Reference strains were*: escherichia coli* (ATCC 25922), klebseilla pneumonie (ATCC700603), pseudomonas aeriginosa(ATCC 9721). *staphylococcus aureus and streptocoque* were obtained from Boudif hopital laboratory of Ouargla.

The agar disc diffusion method was employed for the determination of antimicrobial activity (Changwei et all, 2008). 12 mg of the extract were dissolved in 1 ml of DMSO solvent. Sterile disc of 6 mm diameter (Whatman No.3) was impregnated with extract solution and placed on the previously inoculated agar. The plates were inverted and incubated for 24 h at 37°C. Clear inhibition zones around the discs indicated the presence of antibacterial activity. The assay was carried out in triplicates. A broth micro dilution method was used to determine the minimum inhibitory concentration (MIC) (Changwei et al, 2008). Minimum inhibition concentration (MIC) was determined as described by (Changwei et al, 2008 and Zampini et al, 2005). Different concentrations (37.5-600 µg/ml) of extract were tested. 1 ml of each solution was mixed with 9 ml of Muller Hinton medium and poured into sterilized Petri plates. Immediately after solidification a suspension of the test microorganism (2×10^8 CFU/ml) was spread on the solid media plates. The inoculated plates were incubated at 37◦C for 24 h. The MIC values were determined as the lowest extract or standard concentration at which no growth was observed.

Statistical analysis

Data is expressed as Mean±SD. ANOVA was used to analyze the difference between. P-values of less than 0.05 were considered to be significant.

RESULTS

Phytochemical screening

The phytochemical screening of *Anvillea radiata* showed the presence of flavonoids, Sterols, Steroids, saponins , tannins, Fatty acids and Volatile oils , and the absence of alkaloids (Table1.).

The total phenolics content of the methanolic extract was 572.74±12.7 mg GAE/100g DW of plant with reference to Gallic acid standard curve (y = 3.539X, R^2 = 0.999). The total flavonoid content of the methanolic extract was 72.641± 2.45 mg/100g of Quercetin equivalent of dry weight respectively with reference to standard Quercetin curve (y =37.095X+0.0099, R^2=0.997).

These phytochemical compounds are known to be bioactive compounds and all play a role for antioxidant and antibacterial activities of *Anvillea radiata* extracts.

DPPH radical scavenging activity

DPPH scavenging activity of methanolic extract of *Anvillea radiata*, BHA, BHT and Ascorbic acid is shown in Figure 2.

The IC_{50} values were calculated by the linear regression method of plots of the percent of antiradical activity against the concentration of the tested compounds. The concentration of inhibitors has been calculated in g/l.

In the present investigation, methnolic extract of the *Anvillea radiata*, showed excellent inhibition of DPPH with an IC_{50} of (0.0231) g/l comparable to Ascorbic acid (0.0086), BHA (0.0107) and BHT (0.0137) g/l standard antioxidant drugs used in this study.

Reducing power assay

In the reducing power capacity assay, the extracts cause the reduction of the Fe^{+3} to Fe^{+2} was increased with increasing concentration.Figure2. shows concentration-response curves for the reducing power of the extract, ascorbic acid, BHA and BHT. The sequence for the reducing power was ascorbic acid >BHA >BHT>extract.

Antibacterial activity

Table2. presents diameters of inhibition zones exerted by the methanolic extract towards tested microorganisms. Methanolic extract was effective against the two Gram-positive strains (*S. aureus, Streptocoque*) but no activity was observed against the Gram-negative strain (*Escherichia coli*). Higher inhibition was detected against *Staphylococcus aureus* (Aligiannis et al., 2001) have proposed a classification of plant extract on the basis of their MIC values: strong inhibition: MIC < 500 µg/ml; moderate inhibition: 600 µg/ml < MIC < 1500 µg/ml and low inhibition: MIC > 1600 µg/ml. On the basis of this classification, the methanolic extract of *Anvillea radiata* exert a strong inhibitory activity on *Streptocoque* (MIC = 75 µg/ml).

DISCUSSION

The presence of flavonoids and tannins in *Anvillea radiata* plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds, they are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997).

In the present study, the antioxidant activities were investigated by DPPH and FRAP methods in vitro. Free RSA were decreased in the following order: ascorbic acid >BHA> BHT>Methanolic extract. The methanolic

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extract of *Anvillea. R* is strong radical-scavengers, indicating that active compounds of different polarity could be present in these plants. The high antioxidant activities of this plant might be due to their flavonoid and phenolic contents.

The presence of reductones in the plant extract have been reported in several studies to contribute significantly of the termination of free radical chain reactors as shown in this study (Duh, 1998; Oyedemi, 2011).

Results from this study suggest that phenolic compounds are responsible of the antibacterial activity of methanolic extract of *Anvillea radiata* . Methanolic extract of *Anvillea radiata* was effective against the two Gram-positive strains (S. aureus, *streptocoque*) but no activity was observed against the Gram-negative bacteria (E. *coli*). This is consistent with previous studies reporting that Gram-negative bacteria are more resistant to antimicrobials than Gram-positive microorganisms due to their outer lipopolysaccharide membrane (Khan et al., 2009; Al-Zoreky, 2009; Boulekbache, 2013).

Table 1: Phytochemical constituents of *Anvillea radiata*.: +++: important Presence, ++: average Presence, +: weak presence and -: absence

Composés	Résultats obtenus
Tanins	(+++) Appearance of dark blue color and a precipitate
Flavonoids	(++) Appearance of a red color characteristic aglycone flavones
Sterols et Steroids	(++) color changes from purple to blue or green
Saponins	(++) Appearance of foam shaken in for15 min
Fatty acids	(+) appearance of transparence on filter paper
Volatile oils	(+)
Alkaloids	(-) no precipitate

+++: important Presence, ++: average Presence, +: weak presence and -: absence

Table 2: Antibacterial activity of methanolic extract of *Anvillea radiata*: na: not active, (mean±SD, n = 3)

Bacteria	Gram (+/-)	Zone of inhibition	CMI (µg/ml)
Escherichia coli (ATCC25922)	-	na	na
Klebsiella Pneumonie ATCC70060	-	6.11	300
Pseudomonas aeriginosa ATCC9721	-	7.50	150
Staphylococcus aureus	+	9.20	150
Streptocoque	+	16.48	75

na: not active, (mean±SD, n = 3)

Figure 1: DPPH scavenging effect of Methanolic extract, BHT, BHA and Ascorbic acid. (mean±SD, n = 3)

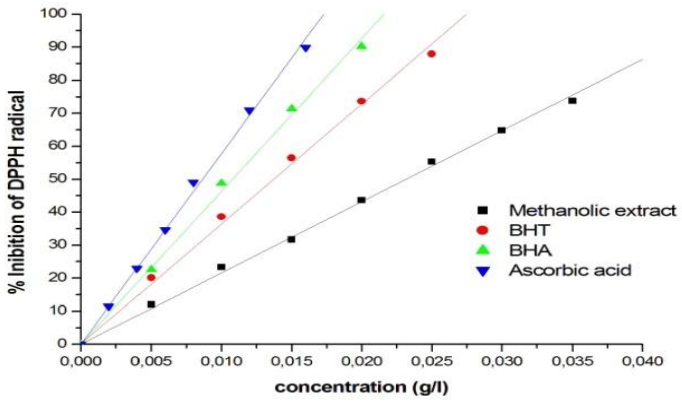
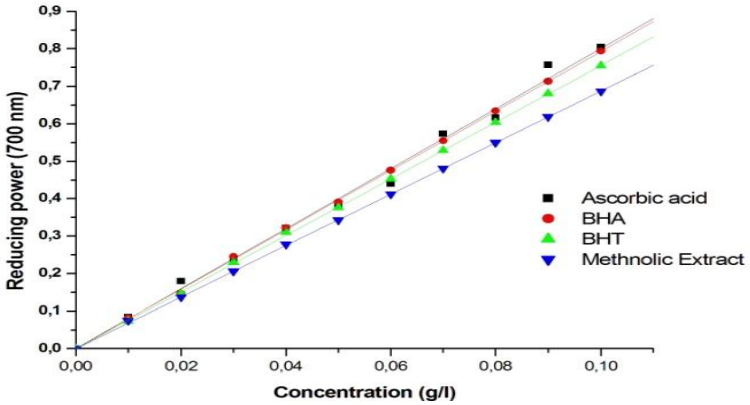


Figure 2: Reducing power effect of Methanolic extract, BHA, BHT and Ascorbic acid. (mean±SD, n = 3)



CONCLUSION

Anvillea radiata has been used as traditional remedies for treatment of various diseases. In this study we report for the first time, the antioxidant and antibacterial activities of methanolic extract of this plant. It may be suggested that the methanolic extract tested possess antioxidant activity which renders them suitable as potential therapeutic. This extract exhibited also strong antibacterial activity against the two Gram-positive strains: *S. aureus* and *Streptocoque*.

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