Phytochemical Study and Antioxidant Activitiesof the Coastal Asteraceae *Achillea Maritima*

Samiha Karih, Amina Abouzid, Rida Nejjari, Mohamed Bakhouch, *Noureddine Mazoir
Laboratory of Bioorganic Chemistry, Research Unit: Valorization of Natural Substances, Chouaib Doukkali University, Faculty of Sciences, PO Box 20, 24000, El Jadida, (Morocco)
***Email:mazoirn@gmail.com**

DOI 10.51129/ujpah-2022-32-1(1)

Received –May 09, 2022
Revised –May 15, 2022
Accepted – May 17, 2022
Published – June 18, 2022

Abstract – This work was focused on the phytochemical study and antioxidant activities of hexane, dichloromethane, ethyl acetate, butanol and aqueous extracts, from the aerial parts of *Achillea maritima*.
The antioxidant activity of *Achillea maritima* extracts was evaluated using the two methods DPPH and FIC. The DPPH activity revealed that ethyl acetate and butanol extracts have an important antioxidant activity at 0.12 and 0.06 mg/mL for the aerial parts of the plant with a percentage of inhibition ranging from 88.29 to 89.72 %, respectively. The results obtained showed that the ethyl acetate extract exhibited a significantly higher ferrous ion chelation activity (83.1%) than the other extracts compared to the EDTA positive control (100%). Dichloromethane and it aqueous extract showed an average capacity for chelation of Fe^{2+} (48.88 % and 45.81 %, respectively). However, the hexane and butanol extracts have a low chelating power (9.5% and 11.3%, respectively).

Keywords: *Achillea maritima*, Organic extracts, Antioxidant activities

Introduction

The human body has a complex system of natural antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a cardiovascular disease (Singh and Jialal, 2006), cancer (Kinnula and Crapo, 2004), neural disorders (Sas et al., 2007), Alzheimer's disease (Smith et al., 2000), mild cognitive impairment (Guidi et al., 2006), Parkinson's disease (Boltonet al., 2000), alcohol induced liver disease (Arteel, 2003).
Plants are potential sources of natural antioxidants. It produces various anti-oxidative compounds to counteract reactive oxygen species in order to survive (Lu and Foo, 1995).
The genus Achillea is widespread all over the world and many species of this genus have been used as traditional herbal medicine.
Among these species, we found *Achillea maritima* (Asteraceae) which is an all-white-cottony plant found on the sands of the Atlantic and Mediterranean coasts.
Therefore, the aim of this study is to evaluate the antioxidative activity of Moroccan Asteraceae *Achillea maritima extracts*, using the two methods DPPH and FIC.

Material and methods

Sampling: *Achillea maritima* was collected at south of El-Jadida city, Morocco (33°14'35"N - 8°32'39"W). The samples were dried at room temperature, desiccated in the open air and protected from the sun's rays, then crushed in order to increase their surface area and facilitate solvent extraction.

Extraction: The samples of the aerial part (200 g) were extracted separately with ethanol (500 mL) for 24 h and the operation was repeated three times in succession. For each part of the plant, the organic phases were combined, dried with Na_2SO_4, filtered and evaporated under reduced pressure. The residue obtained for was solubilized using (200 mL) of distilled water at 100 °C. The mixture was transferred to a separatory funnel and left at room temperature for cooling, and then it was extracted (3x100 mL) with hexane.

The same operations were carried out, increasing the polarity, with the other organic solvents namely dichloromethane, ethyl acetate and butanol (Figure 1).

The four organic phases recovered were dried with anhydrous Na_2SO_4, and then they are filtered, concentrated under reduced pressure.

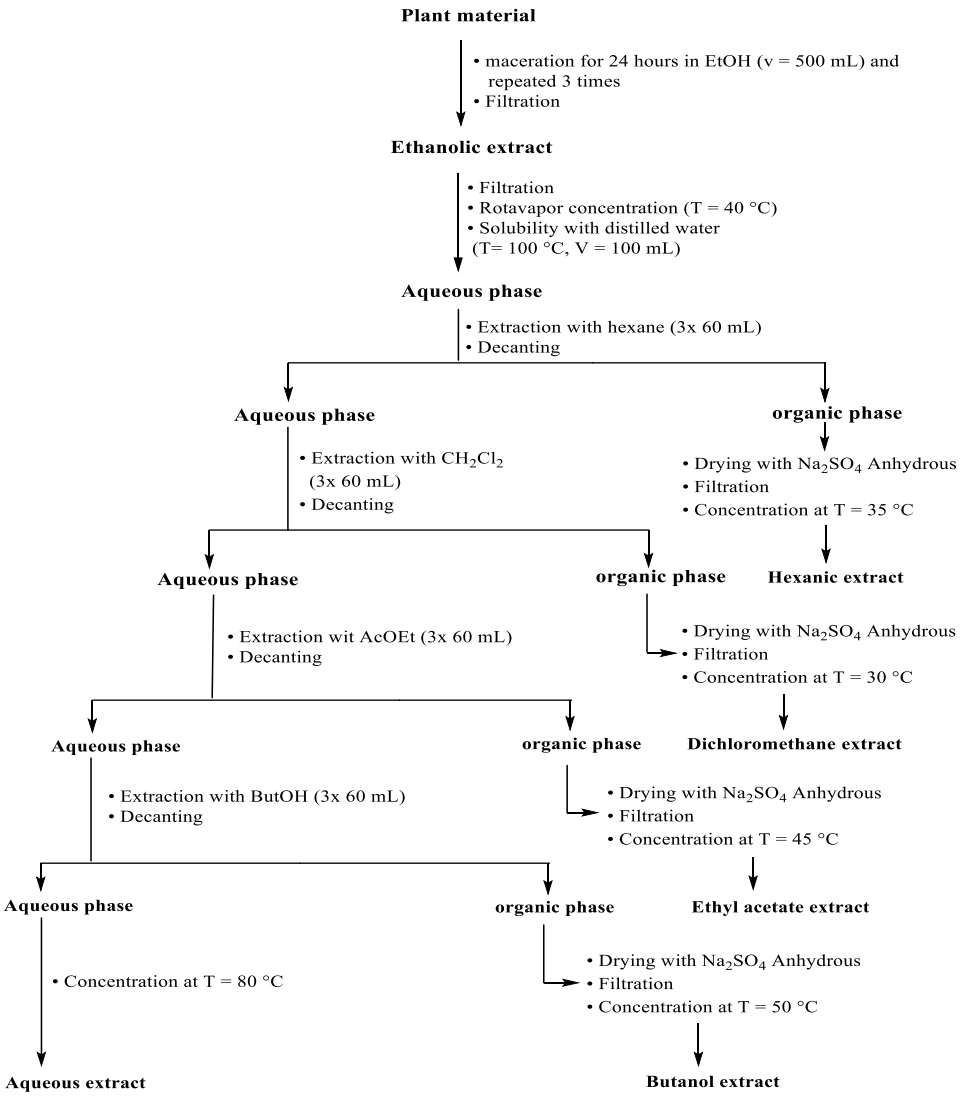

Figure – 1 Extraction protocol to the aerial part of *Achillea maritime*

Antioxidant activities

DPPH radical scavenging activity: The antioxidant activity each extractof *Achillea maritima*was established as diphenylpicrylhydrazyl (DPPH) free-radical scavenging according to the method described (Blois, 1958) with slight modification. DPPH (0.06mM) was dissolved in methanol and added to each extract (with different concentration). The samples were incubated in the dark at room temperature for 30 min. After which the absorbance was measured at 517 nm using a spectrophotometer (UV-Visible Metashe 5200 HPC). The results were compared to a negative control (all reagents except the test extract) and positive controls (BHT and ascorbic acid). The percentage of DPPH radical scavenging was calculated with the following equation: DPPH scavenging activity (%) = [(Ac-As) /Ac] × 100 where Ac is the absorbance of the negative control (methanol with DPPH solution) and as is the absorbance of the sample.

Ferrous Ion-Chelating ability: The iron ion-chelating activity was determined using the method of (Dinis *et al.* 1994), 2.75 m of distilled water was added to1.0 ml of *Achillea maritima*extracts (with different concentration) after which the solution was mixed with 0.05 ml $FeCl_2$ (2.0 mM), 0.2 ml ferrozine (5.0 mM) and. The mixture was shaken vigorously and incubated for 10 min. at room temperature in the dark. The absorbance of the iron ions-ferrozine complex was measured at 562 nm. The ability of each extracts to chelate iron ions was calculated using the following equation: Chelating activity (%) = [1-(Asample – Ablank) / Acontrol] × 100% EDTA was used as the positive control, $FeCl_2$ solution substituted by distilled water was used as a blank, and the sample substituted by distilled water was used as a negative control.

Results and Discussion

DPPH radical scavenging activity: The*In vitro* scavenging ability of *Achillea maritima* extracts compared to the BHT and ascorbic acid are illustrated in Figure 2. From the obtained results, it was shown that ethyl acetate and butanol extracts have an important antioxidant activity at 0.12 and 0.06 mg/mL for the aerial parts of *Achillea maritima* with a percentage of inhibition ranging from 88.29 to 89.72 %, respectively. The values of EC_{50} obtained in DPPH assay for plant extracts are shown together with that of BHT (Table 1). The butanol extracts exhibited the highest radical scavenging activity with low EC_{50} value (0.06 mg/ml) followed by the acetate extract (EC50 = 0.1 mg/mL).

The difference in the radical scavenging activity of *Achillea maritima* could be associated with the nature of theirphenolic compounds. Accordingly, the strong antioxidant property of ethyl acetate and butanol extracts would be associated with their phenols, including the flavonoids which are responsible for their antioxidant effects and also to their free radical scavenging abilities (Zhang and Björn, 2009).

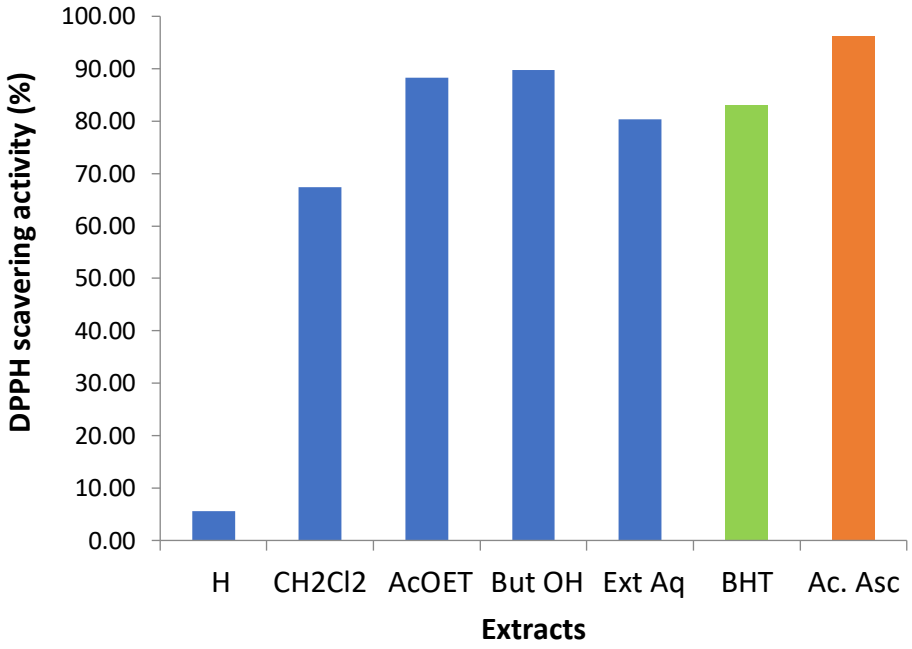

Figure – 2DPPH radical-scavenging activity of hexane (H), dichloromethane (CH_2Cl_2), ethyl acetate (AcOET), butanol (ButOH) and aqueous (Aq Ext) extracts from aerial part of *Achillea maritima* at0.12 mg/mL.

Table – 1DPPH radical scavenging activity (expressed as efficient concentration, EC50) for *Achillea maritima* compared to the BHT.

Extract	DPPH scavenging activity (EC50, mg/ml)
H	16.110
CH_2Cl_2	1.652
AcOET	0.100
ButOH	0.060
Aq	0.370
BHT	0.383

Ferrous Ion-Chelating activity (FIC)

The five organic extracts from aerial part of *Achillea maritima* were tested for their ferrous ion chelating activities. The obtained results showed interesting ferrous ion-chelating ability. Thus, ethyl acetate extract exhibited a significantly higher ferrous ion chelation activity (83.1%) with the EC50 value of 0.03 mg/mL (Table 2) than the other extracts compared to the EDTA positive control (100%). While for the dichloromethane and aqueous extracts showed an average capacity for chelation of Fe^{2+} (48.88 % and 45.81 %, respectively). However, the hexane and butanol extracts have a low chelating power (9.5% and 11.3%, respectively)(Figure 3). It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion(Gordon, 1990). According to (Movahedian *et al.*, 2016), the antioxidant properties of phenolic compounds can be mediated by chelating trace metals involved in free radical production.

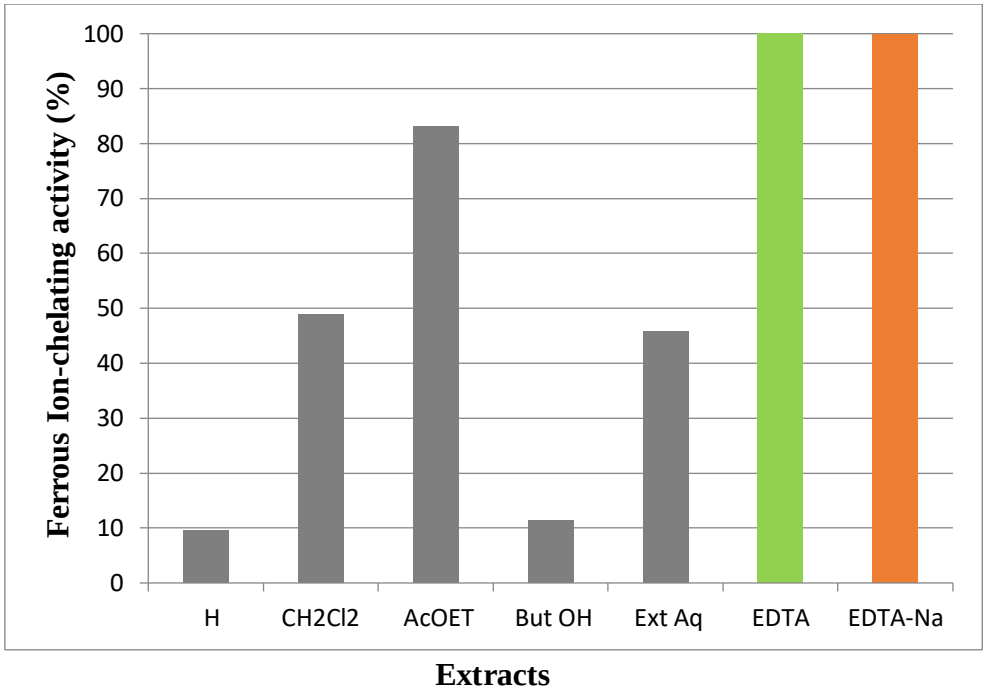

Figure - 3 Ferrous ion chelating activity of hexane (H), dichloromethane (CH_2Cl_2), ethyl acetate (AcOET), butanol (ButOH) and aqueous (Aq Ext) extracts from aerial part of *Achillea maritima* at0.25 mg/mL

Table – 2 Fe^{2+} chelating ability (expressed as efficient concentration, EC50) for *Achillea maritima*

Extract	Fe^{2+} chelating ability (EC50, mg/ml)
H	2.545
CH_2Cl_2	0.283
AcOET	0.033
ButOH	1.152
Aq	0.243
BHT	2.545

Conclusion

In this study, five organic extracts (hexane, dichloromethane, ethyl acetate, butanol and aqueous) from aerial parts of *Achillea maritima*, collected from the Atlantic coasts of Morocco, were tested for their potential antioxidantactivities. The antioxidant activities showed that ethyl acetate and butanol extracts have an important antioxidant activity at 0.12 and 0.06 mg/mL for the aerial parts of *Achillea maritima* with a percentage of inhibitionranging from 88.29 to 89.72 %, respectively. However, ethyl acetate extract exhibited a significantly higher ferrous ion chelation activity (83.1%) with the EC50 value of 0.03 mg/mL.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

References

1. Arteel, G.E. Oxidants and antioxidants in alcohol-induced liver disease. *Gastroenterology*, 2003, 124(3):3778-790.

2. Blois, M.S. Antioxidant determinations by the use of a stable free radical. *Nature*, 1958, 181:1199-1200.

3. Bolton, J.L.; Michael, A.; Trush, M.A.; Trevor, M.; Penning, T.M.; Dryhurst, G. and Monks, T.J. Role of Quinones in Toxicology. *Chem. Res. Toxicol.*, 2000, 13(3):136-160.

4. Dinis, T.C.P.; Madeira, M.C. and Almeida, L.M. Action of Phenolic Derivatives (Acetaminophen, Salicylate, and 5-Aminosalicylate) as Inhibitors of Membrane Lipid Peroxidation and as Peroxyl Radical Scavengers. *Arch. Biochem. Biophys.*, 1994, 315(1):161-169.

5. Gordon, M.H. In: Hudson BJ, (ed.), the mechanism of the antioxidant action in vitro, Food Antioxidants, London: Elsevier, 1990, p_p.1-18.

6. Guidia, I.; Galimbertia, D.; Lonatib, S.; Novembrinoc, C.; Bamontib, F.; Tiriticcoa, M.; Fenoglioa, C.; Venturellia, E.; Barona, P.; Bresolina, N. and Scarpinia, E. Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiol. Aging*, 2006, 27:262-269.

7. Kinnula, V. L. and Crapo, J. D. Superoxide dismutases in malignant cells and human tumors. *Free Radic. Biol. Med.*, 2004, 36(6):718-744.

8. Lu, F. and Foo, L.Y. Toxicological aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK, editors. *Food antioxidants*, 1995, New York: Marcel Dekker.

9. Movahedian, A.; Zolfaghari, B. and Mirshekari, M. Antioxidant effects of hydroalcoholic and polyphenolic extracts of *Peucedanum pastinaci-folium* Boiss. & Hausskn.*Res. Pharm., Sci.*, 2016, 11(5):405-411.

10. Sas, K.; Robotka, H.; Toldi, J. and Vécsei, L. Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *J. Neurol. Sci.*, 2007, 257:221-239.

11. Singh, M. and Jialal, I.Oxidative stress and atherosclerosis. *Pathophysiology*, 2006,13(3):129-142.

12. Smith, M.A.; Catherine A.; Rottkamp, C.A.; Nunomura, A.; Raina, A.K. and Perry, G. Oxidative stress in Alzheimer's disease. *Biochim.Biophys. Acta. Mol. Basis Dis BBAMOL BASIS DIS.*, 2000:139-144.

13. Zhang, W. and Björn, L. The effect of ultraviolet radiation on the accumulation of medicinal compounds in plants. *Fitoterapia*, 2009, 80:207-218