Algerian Journal of Biosciences
ISSN: 2716-9375
Journal homepage: https://www.journal.acse.science/index.php/ajb/index

Original Article

In vitro Antioxidant and anti-inflammatory activities assessment of flavonoids crude extract of *Anthemis pedunculata subsp. atlantica* (Pomel) Oberpr., aerial parts

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ARTICLE INFOR

Article history:
Received 27 September 2023
Revised 15 November 2023
Accepted 18 November 2023

Keywords:
Antioxidant;
anti-inflammatory;
Anthemis pedunculata;
Flavonoids.

ABSTRACT

The Anthemis genus is used in traditional medicine for treating asthma, gastrointestinal disorders, and colds, with its beneficial effects attributed to its phenolic contents. The study aims to evaluate the biological activities of this species, which is widely used by the local population by extracting of flavonoids from Anthemis pedunculata subsp. atlantica (Pomel) Oberpr. and valorised their antioxidant and anti-inflammatory potentials. The extraction process involved macerating the plant in 70% ethanol, defatting it with petroleum ether, and extracting the aqueous phase with chloroform. The flavonoid content was measured using Aluminium chloride solution, while the antiradical activity was assessed by DPPH assay. The study also evaluated the plant extract's reducing power and its ability to protect erythrocytes from membrane lysis induced by hypotonicity using the HRBC membrane stabilization method. The results show a very interesting antioxidant activity expressed by a low IC50 and EC50 in case of DPPH and reducing power test respectively. Also, the extract revealed a significant protective power of erythrocytes at a very low concentration (10−100 µg/mL).

1. Introduction

The family Asteraceae comprises the largest number of described species, belonging to 1600 – 1700 genera [1]. The genus Anthemis (family Asteraceae), known by the common name “chamomile” comprises about 210 species mainly distributed around the Mediterranean region [2]. Anthemis genus (Anthemideae – Asteraceae) species spread in North Africa, West/Southwest and Central Asia and Europe [3].

The vast majority of Anthemis species are consumed as a food flavouring or herbal tea. These species are also of great importance in the cosmetics and pharmaceutical industries [4]. Folk herbal medicine uses Anthemis species mainly for the treatment of gastrointestinal disorders, haemorrhoids, dysmenorrhea, and stomach-ache. Also, members of this genus have been found to possess antibacterial, antispasmodic, anti-inflammatory, hepatoprotective, anticholinesterase, antibiofilm and antioxidant activities [5] [6] [7] [8] [9] [10].The ethnobotanical researches conducted during this study in the north east Algerian's rural communities revealed that this plant was used in traditional medicine for the treatment of asthma, gastrointestinal disorders and colds. It was hypothesized that the positive effects of this plant are attributed to their phenolic contents [11]. Phytochemical investigation of several Anthemis plants revealed the presence of sesquiterpene lactones, polyacetylenes, flavonoids, and essential oils [12].

Botanically, this species is characterized by ribbed achenes. Showing small, scattered tubercles. Flakes of the receptacle progressively acuminate. Plants with spreading-diffuse stems, very rowy, emerging in a tuft from a large vertical stump. Leaves not fleshy leaves not fleshy, pinnatipartite [13].

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Peer review under responsibility of University of El Oued. 2023
DOI : https://doi.org/10.57056/ajb.v4i2.142

This study aims to assess the biological activities of the flavonoids crude extract of Anthemis pedunculata subsp. atlantica (Pomel) Oberpr (the species is in large part utilized by the local populace) grown at an altitude of 1737 m, which makes this plant susceptible to the influence of atmospheric pressure.

2. Materials and Methods

2.1. Plant material

Anthemis pedunculata subsp. *atlantica* (Pomel) Oberpr. aerial parts were collected from the mountain of Megriss of which, the geographic coordinates are X: 5° 18’ 20” Y: 36° 18' 30” and X’: 5° 24' 7” Y’:36° 21’ 54”. Samples were examined and determined in the laboratory of National Institute of Agricultural Research – Setif – Algeria.

2.2. Preparation of extract

The shad dried plant was milled into fine powder and macerated in diluted ethanol (70%) trice (24, 48 and 72 hours), the laboratory temperature (1:10 w/v, 10 g of dried herb), then defatted three times with petroleum ether at 50 C°. The resulted solutions were pooled and concentrated in vacuum to collect the aqueous residue (10 mL). Then, the aqueous phase was extracted with chloroform, acidified with 20% H2SO4 (pH = 5) and treated three time with ethyl acetate. The appearance of an interphase precipitate was observed and only the ethyl acetate fractions were taken as flavonoids crude extract for the experiment [14].

2.3. Determination of total flavonoids content

The flavonoids content in the extract was estimated by the Aluminium chloride solution according to the method described by Bahorun et al., (1996) [15]. Briefly, 1 mL of the methanol solution of the extract was added to 1 mL of 2 % AlCl3 in methanol. After 10 minutes, the absorbance was determined at 430 nm. Quercetin was used as a standard. Results were expressed as mg equivalent Quercetin per gram of extract (mg EQ/GE).

2.4. DPPH Assay

The donation capacity of extract was measured by bleaching of the purple-coloured solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato et al., (1998) [16]. One millilitre of the extract at different concentrations was added to 0,5 mL of DPPH-methanol solution. The mixes were thoroughly mixed before being left at room temperature for 30 minutes in the dark. At 517 nm, the absorbance of the resulting solutions was measured. The antiradical activity was expressed as IC50 (micrograms per millilitre). The ability to scavenge the DPPH radical was computed using the following equation:

DPPH scavenging effect (%) = [(A0 – A1)/ A0]

Where:

A0: the absorbance of the control at 30 min

A1: is the absorbance of the sample at 30 min. Butylated hydroxytoluene (BHT) was used as standard [17].

2.5. Reducing power

The reduction power was calculated using Oyaizu's (1986) approach [18]. A total of 2,5 mL of the extract was added to 2,5 mL of sodium phosphate buffer (pH 6,6; 200 mmol/L) and 2,5 mL of potassium ferricyanide (10 mg/mL). The mixes were incubated for 20 minutes at 50 °C. 2,5 mL of trichloroacetic acid (100 mg/mL) was incorporated after cooling, and the solutions were centrifuged at 200g for 10 minutes. The top layer (5 mL) was diluted with 5 mL of deionized water and 1 mL of ferric chloride (1 mg/mL), and the absorbance at 700 nm was measured against a blank. EC50 value (mg extract/mL) is the effective concentration at which the absorbance was 0,5 for reducing power and was obtained by interpolation from linear regression analysis [19]. Ascorbic acid was used as a reference standard.

2.7. The Human Red Blood Cell (HRBC) membrane stabilization method

The HRBC suspension was prepared with fresh human blood (10 mL) washed three times with normal saline solution and reconstituted as 10 % v/v suspension. The principle was to test the capacity if the extract to protect the erythrocytes from membrane lysis induced by hypotonicity. Briefly, to 1 mL phosphate buffer (pH 7,4; 0,15 M), 2 mL hypo saline (0,36 %), 0,5 mL HRBC suspension (10 % v/v) and 0,5 mL of plant extract or standard drug (DICLOFENAC SODIUM) was added. The mixtures were incubated at 37 °C for 30 minutes then centrifuged at 2500 rpm for 5 minutes. The absorbance of haemoglobin content in the suspensions were read at 560 nm. HRBC membrane haemolysis % may be estimated as follows:

Haemolysis (%) = (Optical density of Test sample / Optical density of Control) × 100.

The percentage of HRBC membrane stability was estimated as follows:

Protection (%) = 100 – [(Optical density of Test sample / Optical density of Control) × 100] [20].

2.8. Statistical analysis

Sample per concentration were used in triplicate and results were expressed as the mean ± standard deviation. Data were statistically analysed with t test of Student and Fisher test with the criterion of P values < 0.05 to determine any significant differences between crude extract of Anthemis pedunculata and standards, using Graph pad prism 8 Demo Software.

3. Results

The carried out extraction method for Anthemis pedunculata gave 1,2 % of flavonoids crud extract contain 13,73±0,62 mg EQ/GE.
Results are showed in figure 1:

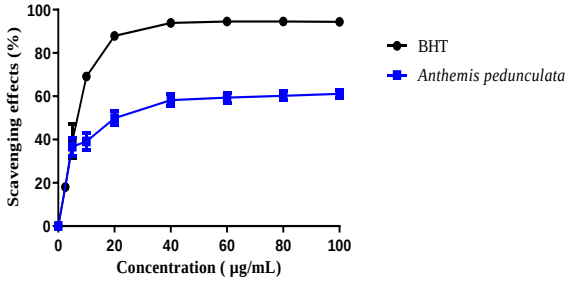


Figure 1: Scavenging effect of *Anthemis pedunculata*

Scavenging activity of the extract reach 61,10±2,03 % at 100 µg/mL against 94,39±04 % at the same concentration. However, an important value of IC 50 reach 26,14±10,73 µg/mL* against 8,76±0,69 µg/mL for standard.

In the case of Reducing power assay, flavonoids crud extract of Anthemis pedunculata (figure 2) an important EC 50 was obtained 27,897±0,460**** µg/mL but weaker compared to the standard 8, 64±0,09 µg/mL.

Hypotonicity induced haemolysis test of Anthemis pedunculata crud extract (10−100 µg/mL) protect significantly the erythrocyte membrane against lysis induced by hypotonic solution (figure 3).

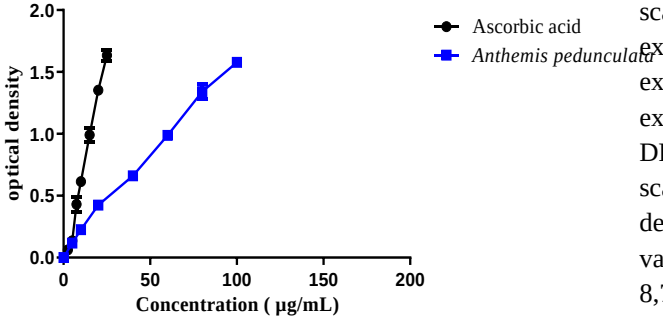
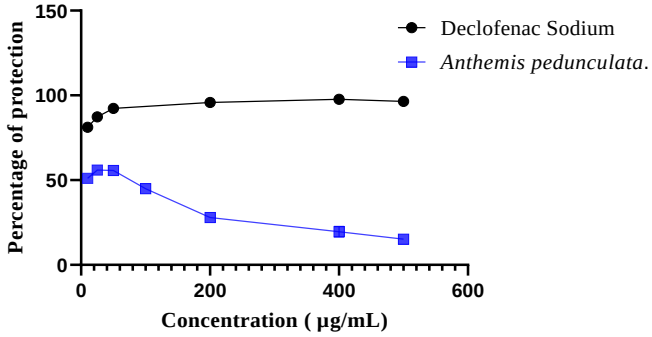


Figure 2: Reducing power assay of standard and *Anthemis pedunculata*.



4. Discussion:

Exploring the flavonoids extract from *Anthemis pedunculata* becomes pivotal not only due to its high-altitude habitat but also because of its widespread use among the local community. Understanding the constituents and potential bioactivities of these flavonoids can offer valuable insights into their medicinal, or therapeutic properties. Furthermore, such research could unveil novel applications, potentially leading to the development of new pharmaceuticals, dietary supplements, or other beneficial products that could positively impact both the local community and broader scientific endeavours.

Oxidative stress plays a pivotal role in the pathogenesis of various metabolic, neurodegenerative, and inflammatory diseases. Free radicals from different biological and environmental sources due to imbalance of natural antioxidants trigger various inflammatory mediators and cause the occurrence of non-alcoholic fatty liver disease, Alzheimer's disease, arthritis, obesity and metabolic diseases which are prevented by administration of natural dietary antioxidants through suppression of the oxidative stress and inflammation [21].

In DPPH assay, the stabilization of DPPH free radicals causes the colour change of reaction solution which is measured by spectrophotometer to determine the scavenging activity of a test antioxidant [22]. In our experience, the IC50 value of Anthemis pedunculata crude extract was 26,14±10.73 g/mL. This suggests that the crude extract may scavenge 50% of the free radicals produced by DPPH at a concentration of 26.14 µg/mL. The extract's scavenging activity at a concentration of 100 µg/mL was determined to be 61,10±2.03%. Nevertheless, the IC50 value for standard BHT (butylated hydroxytoluene) was 8,76±0,69 µg/mL. This means that BHT is a more powerful antioxidant than crude extract since a smaller quantity is required to scavenge 50 % of the free radicals produced by DPPH. At a concentration of 100 µg/mL, BHT had a scavenging activity of 94,39±0,04%. Hence, the antioxidant activity of crud extract of Anthemis

pedunculata revealed a strong antioxidant activity by quenching DPPH free radicals in a dose-dependent manner, but the activity was not stronger than BHT. *Anthemis pedunculata* has exceptional potential, as indicated by its significantly lower IC_{50} value of 26.14 µg/mL when compared to other Anthemis species including *Anthemis cotula*, *Anthemis praecox*, and *Anthemis stiparum*. In compared to *Anthemis cotula*, which has an IC_{50} of 230 µg/mL [23], the potency of *Anthemis pedunculata* is startling. Furthermore, compared to *Anthemis praecox*, which has an IC_{50} of 110 µg/mL [24], and *Anthemis stiparum*, which has an IC_{50} of 92.69 µg/mL [25], *Anthemis pedunculata* has better inhibitory activity, indicating that it is more effective as an antioxidant. The wide range of IC_{50} values emphasizes *Anthemis pedunculata*'s resilience as well as potential antioxidant relevance, making it a suitable candidate for future molecular research.

The antioxidant activity was further proved by measuring the reduction process of ferric (Fe^{+3}) to ferrous (Fe^{+2}) under the action of extract that transformed the yellow colour of test solution to green [226]. The EC_{50} value of a substance is the concentration at which it produces 50% of its maximum effect in a biological assay or experiment. In this case, the flavonoid crude extract has an EC_{50} value of 27,897±0,460 µg/mL, which means that it requires a higher concentration to produce 50% of its maximum effect compared to the standard, which has an EC_{50} value of 8,64±0,09 µg/mL.

Reducing the power of the flavonoid crude extract means that its effectiveness or potency in producing a biological effect would decrease. This could be due to factors such as dilution, degradation, or loss of bioactivity during extraction, processing, or storage.

Therefore, the comparaison of the flavonoid crude extract with the standard found that the extract's EC_{50} value is higher than the standard, this suggests that the extract is less potent or effective than the standard in producing the biological effect you are measuring. However, it's important to note that the results may depend on the specific experimental conditions, the source and quality of the flavonoid extract, and the method used to determine the EC_{50} values.

Membrane stabilization is a process of maintaining the integrity of biological membranes such as erythrocyte and lysosomal membranes against osmotic and heat-induced lysis [27]. Stabilization of erythrocyte membranes exposed to hypotonic induced lysis was employed due to its simplicity and reproducibility. When red blood cells are placed in hypotonic solution in which osmolarity is diminished, the gain in red blood cell water is both instantaneous and quantitative. This phenomenon is put into practical use in the red blood cell osmotic fragility test, which determines the release of haemoglobin from red blood cells in hypotonic sodium chloride (NaCl) solution [28]. Applying the HRBC stabilization membrane assay to the Anthemis pedunculata flavonoids crud extract revealed significant inhibition of haemolysis in response to hypotonic pressure (up to 55% of protection at 25µg/mL). These results revealed that the extract prevents cell membrane lysis and subsequently inhibits inflammation. However, this propriety decreases in increasing concentration and the extract turn to pro-inflammatory substance. This property might be attributed to its phytochemical composition. Notable haemostatic and procoagulant qualities were exhibited by phenolic acids, iridoids, and flavonoids in various bioassays [29].

5. Conclusion

The Anthemis genus is a member of the Asteraceae family with significant importance in the food, cosmetics, and pharmaceutical industries. oxidative stress is a significant contribution to the development of several metabolic, neurological, and inflammatory disorders. The study's focus on Anthemis pedunculata's flavonoid crude extract revealed its potential antioxidant qualities, as evidenced by its DPPH scavenging activity and the reduction process of ferric to ferrous ions. The extract displayed significant antioxidant activity in scavenging free radicals, but with less efficacy than normal BHT (butylated hydroxytoluene). Furthermore, the membrane stabilization experiment demonstrated the extract's capacity to prevent erythrocyte membrane lysis and subsequent inflammation, but with a declining impact at increasing doses. This dynamic behavior could potentially be linked to the extract's phytochemical composition, highlighting the complex nature of its biological impact. While the results underscore the extract's promise in mitigating oxidative stress and inflammation, further investigations are warranted to comprehensively understand its dose-dependent effects, ensuring its optimal use in therapeutic applications and managing potential pro-inflammatory tendencies.

Acknowledgements

Many thanks to Mr. RACHEDI Hamza to his help.

Conflict of Interest

The authors declare that they have no conflict of interest

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Recommended Citation

Gaamoune S. & Nouioua W. In vitro Antioxidant and anti-inflammatory activities assessment of flavonoids crude extract of Anthemis pedunculata subsp. atlantica (Pomel) Oberpr., aerial parts . *Alger. j. biosciences*. 2023, 04(02):096-101.