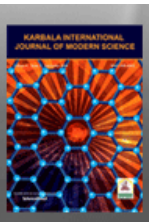

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Total phenolic and flavonoid contents of Anacyclus pyrethrum Link plant extracts and their Antioxidant activity

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Abstract

In this study, *Anacyclus pyrethrm* Link (roots) is a medicinal plant from the Asteraceae family, endemic to Morocco and is widely used in traditional medicine; was investigated for their total phenolics, flavonoids contents and antioxidant activities. The quantitative estimation of total flavonoids and phenols by the colorimetric method showed that the extracts were prepared by fractionation of the crude hydromethanolic extract (ethyl acetate and *n*-butanol). The phenol content of various extracts, estimated by the Folin-Ciocalteu method, was between 1,44 and 12,82 mg EAG / g extract of *A. pyrethrum*. Moreover, it is clear that ethyl acetate was the richest fraction of total phenols in *A. pyrethrum* (12,82 mg EAG / g extract). The determination of flavonoids by the $AlCl_3$ method also revealed that the fraction of ethyl acetate had the highest content (46,82 mg EQ / g of extract).

In addition, we studied the antioxidant activity of the phenolic extracts by the capacity of free radical trapping $DPPH^{\bullet}$. The results reported that the scavenger power of phenolic extracts compared to that of the standard antioxidant, ascorbic acid (IC_{50} = 0,051 mg / ml), allowed us to deduce that the antioxidant power of all extracts tested by *A. pyrethrum* studied had an important antioxidant power. The IC_{50} values were classified in the following order of antioxidant potency: ethyl acetate fraction (0,144 mg / ml) > hydromethanolic extract (0,152 mg / ml) > butanolic extract (0,155 mg / ml).

The results indicated that the plants tested may be potential sources for isolation of natural antioxidant compounds.

Keywords

Anacyclus pyrethrum, Total phenolic content, flavonoid content, DPPH•, Antioxidant activity.

Cover Page Footnote

Acknowledgements We are grateful to Mr M. Ibn Tattou, Professor at the Scientific Institute of Rabat, for the species identification.

1. Introduction

For a long time, natural products, especially those of plant origin, have always been an important source of raw material essential for the discovery of a huge variety of bioactive molecules with multiple interests put to use in the food, cosmetics and pharmaceutical industries [1].

Nowadays, there is a growing interest in the biology of free radicals. The world of biological and medical sciences is invaded by a new concept, that of "oxidative stress". This is a situation where the cell no longer controls the excessive presence of toxic oxygen radicals; it will have to protect itself from these excesses by different antioxidant systems. Currently, it is well recognized that although oxidative stress is not a disease in itself, it is potentially involved in many pathologies [2]. It should be noted that oxidants, whatever their origin, constitute a very serious problem not only in public health but also in the agri-food industry. Antioxidant molecules of natural origin have attracted considerable research attention and have created a new Area in the exploitation of secondary metabolites in human health as well as in the food industry. Phenolic compounds form a very large set of substances that are difficult to define easily. The high efficiency of these substances in stopping radical reactions by neutralizing free radicals is mainly due to the phenolic structure with the presence of free hydroxyl groups or engaged in another functional groups (ether, ester, heteroside …) [3−8].

In this context, the aim of this study was to contribute to the valorization of the Moroccan Middle Atlas flora in order to discover new therapeutic agents. Thus, we have considered performing an assay of total phenols and flavonoids of the roots of a spontaneous and endemic species of Morocco, *Anacyclus pyrethrum* L., in order to evaluate the antioxidant activity of its extracts, using the technique of DPPH (2,2-diphenyl-1-picrylhydrazyl).

2. Materials and methods

2.1. Plant material

Wild roots of *A. pyrethrum* were collected, during April 2012, from the Timahdite region (33° 14′ 13″ N; 5° 03′ 36″ W at 1800 m altitude) that is located in the Moroccan Middle Atlas with a semi-arid climate and characterized by an average rainfall of 695 mm/year. Afterward, they were dried in the shade to be protected from humidity at room temperature for 10 days before their harvest. A botanical identification of the species was carried out by Professor Mohamed Ibn Tattou of the National Herbarium at the Rabat Scientific Institute (Morocco). Supporting specimens and voucher numbers have been deposited at the Herbarium of the same Institute.

2.2. Extraction of phenolic compound

The solid-liquid extraction of the total phenols and flavonoids was carried out by macerating of 30 g of crushed dry sample sprayed in 300 ml of aqueous methanol solution (70%) under gentle stirring at room temperature during 48 h. After filtration and vacuum concentration, the crude residue is fractionated according to the Bruneton's (1993) method with a slight modification [9]. It was collected in hot water. The aqueous phase underwent successive liquid-liquid extractions (fractionation) using organic solvents of increasing polarity (ethyl acetate and *n*-butanol). The organic and aqueous phases thus obtained were concentrated to dryness under reduced pressure in a rotary evaporator and the dry residues were taken up in distilled water and then stored at 4°C until they were used.

2.3. Dosage of total phenols of plant extracts

The total phenol content of the extracts was determined by Folin-Ciocalteu reagent. It was carried out by a method adapted from Abbas Ali Dehpour et al. (2009) [10]. In a 100 ml volumetric flask, 200 µl of each extract is mixed with 1.5 ml of 10% Folin-Ciocalteu reagent. The mixture was stirred and allowed to stand for 6 min before the addition of 1,5 ml of 7,5% (w/v) aqueous sodium carbonate solution Na_2CO_3. Then, the solutions were adjusted with distilled water to obtain a final volume of 100 ml. The reaction mixture is homogenized immediately and kept in the dark for 2 h at room temperature. The absorbance reading of each solution was determined at 765 nm with a Shimadzu UV-MINI 1240 spectrophotometer. A calibration curve was performed by adopting the same operating procedure and using gallic acid as a positive control. The total phenol concentrations of each extract were calculated from the regression equation of the

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calibration range established with gallic acid (y = 0,095x + 0,003). The results are expressed in milligrams of gallic acid equivalent per gram of dry matter (mg EAG/g of dry plant).

2.4. Dosage of flavonoids in plant extracts

The flavonoids were quantified by the direct dosing with aluminum trichloride according to a method adapted from Djeridane et al. (2006) [11]. In a 50 ml volumetric flask, 100 µl of each extract is mixed with 20 ml of distilled water. After 5 min, 100 µl of 10% (m/v) aluminum trichloride ($AlCl_3$) is added. The solutions were then adjusted to 50 ml with pure methanol, homogenized immediately and then kept in the darkness for 30 min at room temperature. The absorbance of each solution was determined at 433 nm with a spectrophotometer cited above. A calibration curve is performed by adopting the same procedure and quercetin is used as a positive control. The flavonoid concentrations of each extract were calculated from the regression equation of the calibration range established with quercetin (y = 0,073x − 0,081). The results are expressed in milligrams of quercetin equivalent per gram of dry matter (mg EQ/g of dry plant).

2.5. Evaluation of antioxidant activity by DPPH$^{\bullet}$ free radical scavenging method

The experiment was carried out using the spectrophotometric quantification method cited above. The solution of DPPH (1,1-diphenyl-di-picrylhydrazyl) at $6{,}10^{-5}$ M is obtained by dissolving 2,4 mg of the powder in 100 ml of ethanol. The samples to be tested were prepared by dissolution in ethanol at a rate of 1,6 mg/ml [12]. The test is performed by mixing a volume of 2,8 ml of the previous solution of DPPH with 200 µl of extracts of our samples or standard antioxidant (ascorbic acid) at different concentrations (0–200 µg/ml). After 30 min of incubation in the darkness and at room temperature, the absorbance is read at 515 nm against a blank that contains only ethanol. The anti-radical activity is expressed as the percentage of reduction or inhibition (I %) of free radical DPPH according to the following equation [13,14]:

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

I (%): Percentage of inhibition.Abs control: Absorbance of the solution containing only the solution of the radical DPPH.Abs sample: Absorbance of the solution of the samples to be tested in the presence of DPPH.

The IC_{50} were determined graphically from the polynomial trend curves of 3rd degree plotted, representing the inhibition percentages as a function of the different doses of the tested fractions. IC_{50} values are expressed in mg/ml, which is inversely related to antioxidant capacity [15].

2.6. Statistical analysis

The experimental data obtained were expressed as an average. The statistical analysis of the data, including the correlation coefficient of the antioxidant properties, was carried out using Excel software (Microsoft Office 2010).

3. Results and discussions

3.1. Yield of phenolic extracts

Various extracts were obtained by solid-liquid maceration of the phenolic compounds in a hydromethanolic solution (70%) followed by fractionation with pure solvents of increasing polarity (ethyl acetate and *n*-butanol). This allowed us to determine the yields (Fig. 1) of different extracts (fractions) in relation to the weight of the dry matter and also in relation to the hydroethanolic extract (crude).

Examination of the obtained results allowed highlighting that the *A. pyrethrum* contained a remarkable content of extractable compounds. Thus, it was clear that the use of solvents of different polarities made it

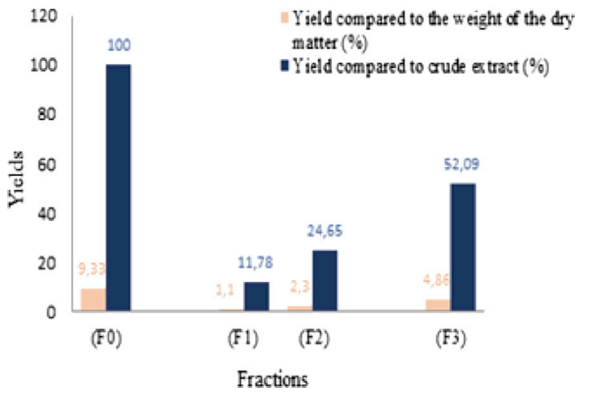

Fig. 1. Yields of different root extracts of Anacyclus pyrethrum. (F0) Crude extract; (F1) Ethyl acetate Fraction; (F2) n-Butanol fraction; (F3) Aqueous fraction

possible to separate the different metabolites found according to their degree of solubility in the extraction solvent, and according to their structural complexity [6,16,17].

Of the four fractions, that hydromethanolic represented the highest yield in *A. pyrethrum* (9,33%), relative to the dry matter weight (Fig. 1). On the other hand, the comparison between the yields of different other fractions compared to the crude extract of the studied species, indicated that the content extractable compounds decreased in the decreasing of the solvents polarity used F3, F2, and F1 respectively (52,09%, 24,65%, and 11,78%). In addition, the sum of the yields of these fractions was estimated at 88,52%. Given the relatively high number of washes performed for the separation of the three fractions or fractionation of the crude extract which can lead to substantial losses of material, these losses represent in the species studied 11,48% of extractable compounds.

In previous studies, the methanolic macerate of *Anacyclus clavatus* flowers yielded about 6,78 ± 0,23% [18]. Whereas, the *A. pyrethrum* ethanolic macerate, from Spain and India, recorded yields of 4,34% and 11,53% respectively [19,20]. In fact, rare works mentioned the yields of the different fractions prepared according to our methodology.

3.2. Quantitative analysis of total phenols and flavonoids of plant extracts

In order to characterize extracts prepared from the roots of *A. pyrethrum*, quantitative analyzes of the total phenols are determined from the linear regression line equation of the calibration curve and are expressed in milligram equivalent of gallic acid per gram of dry matter or extract (mg EAG/g) (Fig. 2). Thus, flavonoids were also determined from the equation of the linear regression line of the calibration curve and are expressed in milligram equivalent of quercetin per gram of dry matter or extract (mg EQ/g) (Fig. 2).

Fig. 3 summarized the results obtained based on the absorbance values, by a UV−Visible spectrophotometer, relating to the total phenol and flavonoid contents of the crude extracts hydromethanolic (F0), ethyl acetate (F1), *n*-butanol (F2) and the residual aqueous phase (F3) of the *A. pyrethrum* roots.

The quantitative determination of the total phenols and flavonoids of the species studied in the different phases obtained clearly shows that almost all the phenolic compounds present in the crude extract are exhausted by organic solvents and distributed in the

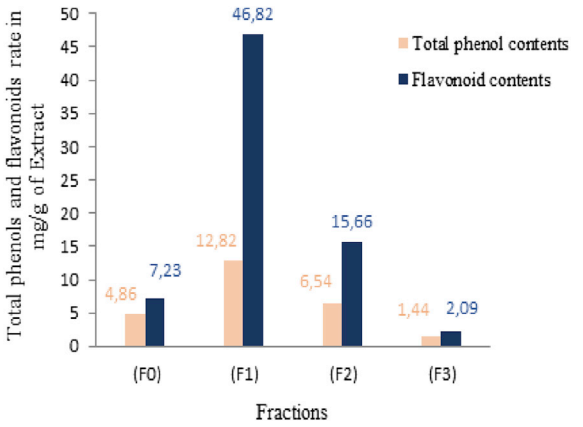

Fig. 3. Quantity of phenolic compounds and flavonoids from *A. pyrethrum* extracts. (F0) Crude extract; (F1) Ethyl acetate Fraction; (F2) n-Butanol Fraction; (F3) Aqueous fraction.

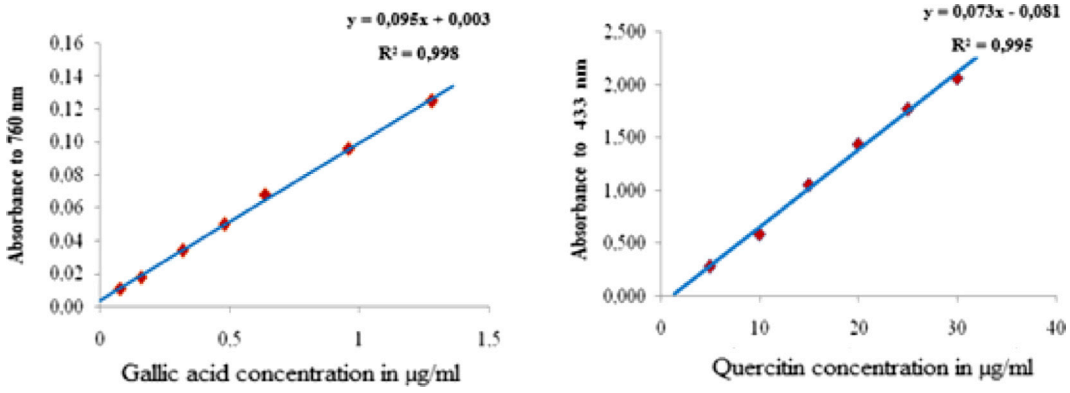

Fig. 2. Calibration line for gallic acid and quercetin.

ethyl acetate and *n*-butanolic fractions during fractionation. This is confirmed by the low values recorded in the residual aqueous fraction. Indeed, according to the results, all *A. pyrethrum* extracts (F0; F1; F2; F3) are richer in flavonoids (7,23; 46,82; 15,66; 2,09 mg EQ/g extract respectively) than in total phenols (4,86; 12,82; 6,54; 1,44 mg EAG/g extract). Similar results have been reported by Talbi et *al*. (2015) and Meziti (2009) [7,21].

3.2.1. Dosage of total phenols

Reproductivity was obtained for the dosage of total phenols because the measured absorbance is clearly correlated with the concentration of gallic acid used in the standard range ($y = 0{,}095, x + 0{,}003, R^2 = 0{,}998$).

The total phenolic compound contents of the *A. pyrethrum* roots were notable. It was clear that ethyl acetate was the richest fraction of total phenols (12,82 mg EAG/g extract), followed by the *n*-butanolic fraction (6,54 mg EAG/g extract), the crude extract (4,86 mg EAG/g extract) and the residual aqueous fraction (1,44 mg EAG/g extract).

Compared to other studies, the methanolic and aqueous extracts of the aerial parts of pyrethrum (from Algeria) have a polyphenol content of 310,78 ± 5,2 and 183,82 ± 3,1 mg EAG/g of extract respectively [22]. It also seems that the hydromethanolic extracts of the aerial and subterranean parts respectively of *A. clavatus* (Algeria) and *A. pyrethrum* (India) had a phenol content of 71,09 ± 3,84 and 62,89 ± 0,43 mg EAG/g extract respectively [23,24]. As a reminder, ethanolic extracts from roots of the same species, *A. pyrethrum* from India and Spain, were found to be rich in total polyphenols estimated at 159,63 ± 0,52 and 42,21 ± 1,59 mg EAG/g extract respectively [19,25].

Generally, high content of phenolics compounds compared to flavonoids are logical since flavonoids were the major polyphenol compounds. This indicates that the extracts contain other phenolic compounds with chemical structures other than flavonoids (phenolic acids, tannins, …) [26]. This can also be explained by an increase in the phenolic metabolism of the plant and by the existence of a link with the harsh climatic conditions and growing conditions such as high temperatures, the duration of solar exposure, soil nature, drought, salinity and growing season [11,27].

It is evident that this variability in the results of the total phenolic compound content measured by the Folin-Ciocalteu process does not give a complete picture of the quality or quantity of the phenolic compounds in the extracts [8,28]. The low specificity of the Folin-Ciocalteu reagent is the main disadvantage of the colorimetric assay [29]. The reagent is extremely sensitive to the reduction of all hydroxyl groups not only those of phenolic compounds, but also those of certain non-phenolic substances such as sugars, proteins, sulfur compounds, dyes, etc. These groups can thus interfere during any phenolic evaluation. They are also likely to polymerize with phenolic compounds, which leads to the formation of complexes that are not detected by the test used [17,27,30–33]. The assay by this reagent therefore gives a crude evaluation of all the phenolic compounds of an extract. It is not specific to polyphenols, but many compounds may react with the reagent, giving a high apparent phenol level [33–35].

3.2.2. Dosage of flavonoids

Examination of the flavonoid quantification results shows a positive correlation between the quercetin concentration used as standard and the absorbance with a correlation coefficient of $R^2 = 0{,}995$. This flavonoids level varied considerably across the various measured extracts and spread in pyrethrum from 2,09 to 46,82 mg EQ/g of extract. As for the total phenolic compounds, the ethyl acetate fraction also has a high flavonoids content in the species studied (respectively 46,82 and 31,85 mg EQ/g of extract), followed by the n-butanolic fraction (15,66 and 17,15 mg EQ/g extract), the crude extract (7,23 and 10,76 mg EQ/g extract) then the residual aqueous fraction (2,09 and 2,61 mg EQ/g extract). Taking into account the selectivity of each solvent used for the fractionation where the ethyl acetate is the most selective, it is assumed that the species studied is rich in flavonoids aglycones or flavonoids mono-O-glycosides and partially in di- O-glycosides [36,37]. The n-butanol phase recovered in particular flavonoids di and triglycerides. The final aqueous phase contains mainly the more polar glycosylated flavonoids [36].

Also, the researches continuing on the species of *A. pyrethrum*, in 2012, Selles et *al*. report that the aerial parts of the species originating in Algeria have variable flavonoid contents: the methanolic and aqueous extracts have contents of 92,50 ± 4.2 and 72,50 ± 2,1 mg EQ/g of extracted respectively [22]. The works conducted by Sujith et *al*. (2011) and Tuekaew et *al*. (2014) revealed a flavonoid content of 29,65 ± 0,83 and 7,74 ± 0,18 mg EQ/g extract in the ethanolic extracts of *A. pyrethrum* roots from India and Thailand respectively [19,25]. Also the hydromethanolic extracts of the aerial and underground parts of *A. clavatus* species from Algeria and *A. pyrethrum* from India have flavonoid contents of 3,60 ± 0,25 and 38,89 ± 0,52 mg EQ/g extract respectively [23,24].

The difference in polyphenols levels (including flavonoids) in the extracts of the species studied can be attributed to intrinsic factors (genetic factors) and extrinsic factors (such as geographical factors, climatic factors that stimulate the biosynthesis of these compounds, also the degree of plant maturity and storage duration) [38]. In addition, the polyphenols dosing is conditioned by several factors including the extraction method, the standard and the quantification method.

3.3. Scavenging effect of the free radical DPPH• by organic plant extracts

Absorbance measurement (or optical density OD) was performed by UV–Visible spectrophotometry at 515 nm. From the values obtained, we calculated the percentages of inhibition using the formula given previously. The values obtained made it possible to plot the the trend curves which represent the variation of the percentage of inhibition as a function of the concentrations. The percentage inhibition increases gradually or progressment until reaching a plateau that corresponds to the almost total containment of the DPPH present in the medium. The reduction of this radical is accompanied by its passage from the violet color (2.2 diphenyl-1-picryl hydrazyl (DPPH•)) to the yellow color (2,2 diphenyl -1-picryl hydrazine (DPPH-H)) [21,39].

From the curves of the inhibition rates (figures given below) of the different plant extracts, as well as that of the standard, we were able to graphically deduce the IC_{50} values. Analysis of antioxidant activity results was evaluated by comparison of the IC_{50} values; the lowest value corresponded to the highest efficiency [40].

According to the results of the antioxidant activity of the organic extracts of the studied species, evaluated by the DPPH test, we noted that the percentage of inhibition of the free radical increased with the increase of the concentration as well for the vitamin C used as a positive control that for the different extracts tested (Fig. 4).

In fact, the results found (Fig. 5) recorded IC_{50} of about 0,155 mg/ml for the butanolic fraction (F2); 0,152 mg/ml for the hydromethanolic extract (F0) and 0,144 mg/ml for the ethyl acetate phase (F1). The high activity is attributed to the fraction of ethyl acetate, it has the lowest value of IC_{50} which is also quite close to that of vitamin C (0,051 mg/ml), whose reactions with the DPPH• are quite fast.

Moreover, in the bibliography several studies have been carried out on the antioxidant activity of different organic extracts of the genus *Anacyclus*. The work of Krimat et *al*. (2014), Aliboudhar and Tigrin-Kordjani (2014) successively reported that the hydromethanolic and methanolic extracts of the aerial parts of *A. clavatus* from Algeria exerted a powerful anti-radical effect, evaluated by IC_{50} of 0,027 ± 0,80 and 0,125 ± 3,61 mg/ml respectively [18,23]. Also, the methanolic and aqueous extracts of the aerial parts of *A. pyrethrum* from Algeria had a strong capacity to trap free radicals given their IC_{50} of the order of 0,056 mg/ml and 0,114 mg/ml respectively [22]. The antioxidant activity of the ethanolic extract (80%) of *A. pyrethrum* from Lagasca was actually less important (IC_{50} = 0,300 ± 7,51 mg/ml) [19]. On the other hand, the studies carried out by Kakatum (2011) and Sujith et *al*. (2011), via antioxidant tests of the ethanolic extract of the roots of *A. pyrethrum* from India, rise on very interesting results with IC_{50} of the order of 0,0239 ± 1,36 and 0,055 mg/ml respectively [20,25]. The evaluation of the antioxidant properties of the ethyl acetate and butanolic fractions, by the DPPH test according to our methodology, was reported by several researchers working on many other plant species [3,37,41].

According to the results, the antiradical activity of plant extracts, evaluated by simple tests, was only due to a production of secondary metabolites [42]. It was relatively dependent on the content of total polyphenols and flavonoids. In another way, the examination of the results allowed us to distinguish heterogeneity in the correlation that linked IC_{50} values and the total phenol or flavonoid contents. These results were expected because according to the recorded values, there was a relative dependence between the antioxidant activity and the contents of the total polyphenols and flavonoids. For example, the ethyl acetate extract, in the species studied which represents the high fraction of polyphenols and flavonoids, has a greater trapping effect than the other extracts. If we compare the contents of total phenols and flavonoids in crude and butanolic extracts, it appears that the extract, which is richer in polyphenols or flavonoids, has a lower antioxidant power. This can be explained by the presence of metabolites in the crude extract that augmente antioxidant activity. In fact, the phenolic fraction does not incorporate all the antioxidants and the synergistic interactions between the antioxidants in a mixture makes that the antioxidant activity depends not only on the concentration (quantitative), but also on the structure and nature of the antioxidants (qualitative). These results are similar to those of Falleh et *al*. (2008), Czapecka et *al*. (2005) and Salih (2013) [43–45]. Different results have been reported in other

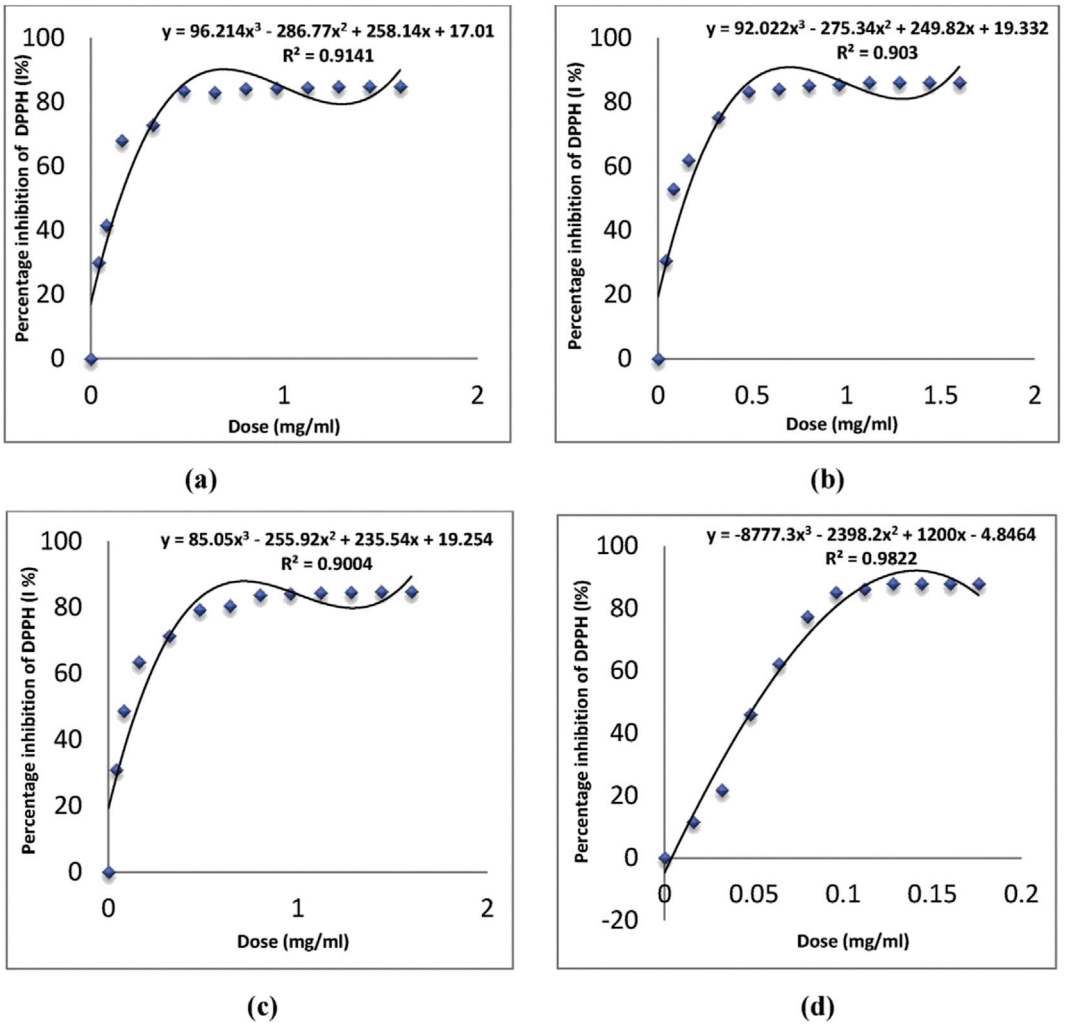

Fig. 4. Percentage inhibition of the free radical DPPH$^{\bullet}$ as a function of different concentrations used extracts of *Anacyclus pyrethrum* (crude (a), ethyl acetate (b), butanolic (c)), and that of ascorbic acid (d).

studies, whose correlation between antioxidant activity and phenol totaux and flavonoid levels was strong [8,11,46].

Likewise, the results found may be influenced by the selected total phenol assay method. In our case, it is the Folin-Ciocalteu method, which does not give an exact qualitative or quantitative image of the total phenol content [8,28,47]. In fact, the mechanism of the reaction between antioxidant and DPPH depends on the structural conformation or spatial configuration and the number of OH groups of the antioxidant [48]. Some compounds reacted very quickly with DPPH by reducing a number of DPPH molecules equal to that of the hydroxyl groups of the antioxidant. Then a thorough study is necessary to characterize the molecules responsible for the activity and consequently to establish an activity-chemical structure relationship, in other words the bio-activity of these active ingredients.

4. Conclusion

The results of this work suggested the importance of *A. pyrethrum* as a reliable source of active ingredients known for their therapeutic properties. The dosage of the different phases obtained, extracted from the roots of *A. pyrethrum* endemic to the Moroccan Middle Atlas, revealed that they were rich in total phenols and flavonoids. The ethyl acetate fraction had the highest content of total phenols and flavonoids, natural antioxidant substances of considerable interest in

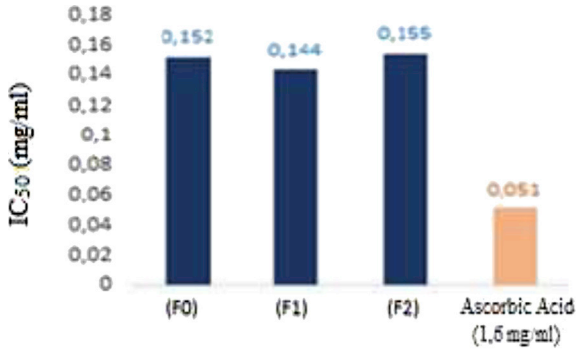

Fig. 5. IC_{50} values Illustration of different extracts of *A. pyrethrum*, and ascorbic acid.

pharmacology. Preliminary results of the DPPH test of the species studied revealed that their tested extract samples have shown *in vitro* an antioxidant activity. In addition, the organic ethyl acetate extract had a higher activity. However, this activity was more pronounced in pyrethrum. This difference in activity can be related, sometimes quantitatively or qualitatively, to the phenolic compounds present in the plant extract. Finally, all of these results obtained *in vitro* were only a first step in the search for biologically active substances and natural sources. To valorize this plant, additional tests (the isolation and the identification of the bioactive compounds) will be necessary and should be able to confirm the found results.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

[1] T. Bahorun, B. Gressier, F. Trotin, C. Brunet, T. Dine, M. Luyckx, J. Vasseur, M. Cazin, J.C. Cazin, M. Pinkas, Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations, Arzneim. Forsch. 46 (1996) 1086−1089.

[2] E. Guinebert, P. Durand, M. Prost, R. Grinand, R. Bernigault, Mesure de la résistance aux radicaux libres, Sixièmes Journées de la Recherche Avicole, 2005, pp. 554−558.

[3] N.B.N. Belyagoubi, Activité Antioxydante Des Extraits Des Composés Phénoliques De Dix Plantes Médicinales De L'ouest Et Du Sud-Ouest Algérien, Université Aboubakr Belkaïd-Tlemcen, Algerie, 2011.

[4] M.M.R. Kansole, Etude Ethnobotanique, Phytochimique Et Activites Biologiques De Quelques Lamiaceae Du Burkina Faso: Cas De *Leucas Martinicensis* (Jacquin) R. Brown, *Hoslundia Opposita* Vahl et *Orthosiphon Pallidus* Royle Ex Benth, Universite De Ouagadougou, Burkina Faso, 2009.

[5] A. Cakir, A. Mavi, A. Ytldirim, A. Duru, M.E. Harmandar, M.C. Kazaz, Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopfolium* L. by activity-guided fractionation, J. Ethnopharmacol. V (2003) 73−83.

[6] F. Belkhiri, Activité antimicrobienne et antioxydante des extraits du Tamus communis L. et *Carthamus caeruleus* L., Université farhat abbes- setif-, Alg2rie, 2009.

[7] A. Meziti, Activité Antioxydante Des Extraits Des Graines De *Nigella Sativa* L Étude In Vitro Et In Vivo. Universite El-Haj Lakhdar Batna Faculté des Sciences, Universite El-Haj Lakhdar Batna, Algérie, 2009.

[8] A. Wojdylo, J. Oszmianski, R. Czemerys, Antioxidant activity and phenolic compounds in 32 selected herbs, Food Chem. 105 (2007) 940−949.

[9] J. Bruneton, Pharmacognosie, Phytochimie, Plantes Médicinale, 2éme Edition, Édition Tec Et Doc, Paris, France, 1993.

[10] A.A. Dehpour, M.A. Ibrahimzadeh, N. Seyed Fazel, M.N. Seyed, Antioxydant activity of the methanol extract of *Ferula Assafoetida* and its essential oil composition, Grasas Aceites 60 (2009) 405−412.

[11] A. Djeridane, M. Yous, B. Nadjemi, D. Boutassouna, P. Stocker, N. Vidal, Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds, Food *Chem.* 97 (2006) 654−660.

[12] F. Nikhat, D. Satynarayana, E.V.S. Subhramanyam, Isolation, charectrisation and screening of antioxidant activity of the roots of *Syzygium cuminii* (L) Skeel, Asian J. Res. Chem. 2 (2009) 218−221.

[13] F. Sharififar, G. Dehghn-Nudeh, M. Mirtajaldini, Major flavonoids with antioxidant activity from *Teucrium polium* L, Food Chem. 112 (2009) 885−888.

[14] A. Braca, C. Sortino, M. Politi, I. Moreli, J. Mendez, Antioxidant activity of flavonoids from *Licania Licaniaeflora*, J. Ethnopharmacol. 79 (2002) 379−381.

[15] M. Jukié, M. Milos, Catalytic oxidation and antioxidant properties of the thym essential oils (*Thymus vulgareae L.*), Croat. Chem. Acta 78 (2005) 105−110.

[16] A. Kar, Pharmacognosy and Pharmacobiotechnology second ed., vols. 1−30, New Age International (P) Limited, Daryaganj, New Delhi, 2007, pp. 122−777, 70.

[17] S. Vuorela, Analysis, Isolation, and Bioactivities of Rapeseed Phenolics (Dissertation). EKT Series 1343, University of Helsinki, Fonlande, 2005. ISSN 0355-1180.

[18] H. Aliboudhar, N. Tigrine-Kordjani, Effect of extraction technique on the content and antioxidant activity of crude extract of Anacyclus clavatus flowers and their essential oil composition, Nat. Prod. Res. 28 (2014) 2140−2149.

[19] J. Tuekaew, N. Siriwatanametanon, Y. Wongkrajang, R. Temsiririrkkul, I. Jantan, Evaluation of the antioxidant activities of Ya-hom intajak, a Thai herbal formulation, and its component plants, Trop. J. Pharm. Res. 13 (2014) 1477−1485.

[20] N. Kakatum, Inti-inflammatory Activity of Thai Traditional Remedy Extract for Muscle Pain Treatment Called Sahasthara and its Plant Ingredients, University Thammasat, Thailand, 2011. Thesis.

[21] H. Talbi, A. Boumaza, K. El-mostafa, J. Talbi, A. Hilali, Evaluation de l'activité antioxydante et la composition physico-chimique des extraits méthanolique et aqueux de la *Nigella sativa* L. J. Mater. Environ. Sci. 6 (2015) 1111−1117.

[22] Ch Selles, D.M. El Amine, H. Allali, B. Tabti, Evaluation of antimicrobial and antioxidant activities of solvent extracts of *Anacyclus pyrethrum* L., from Algeria, Mediterr. J. Chem. 2 (2012) 408−415.

[23] S. Krimat, T. Dob, L. Lamari, S. Boumeridja, C. Chelghoum, H. Metidji, Antioxidant and antimicrobial activities of selected medicinal plants from Algeria, J. Coast. Life Med. 2 (2014) 478−483, https://doi.org/10.12980/JCLM.2.2014APJTB-2014-0071.

[24] M.D. Kalim, D. Bhattacharyya, A. Banerjee, S. Chattopadhyay, Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine, BMC Complement Altern. Med. 10 (2010) 1−11.

[25] K. Sujith, C. Ronald darwin, V. Suba, Antioxidant activity of ethnolic root extract of *Anacyclus pyrethrum*, Int. Res. J. Pharm. 2 (2011) 222−226.

[26] B. Bakchiche, A. Gherib, Activités antioxydantes des polyphenols extraits de plantes médicinales de la pharmacopée traditionnelle d'Algérie, Int. J. Innov. Appl. Stud. 9 (2014) 167−172.

[27] N. Boizot, J.P. Charpentier, Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier, Le Cahier des Techniques de l'INRA, 2006, pp. 79−82.

[28] T. Katsube, H. Tabata, Y. Ohta, Y. Yamasaki, E. Anuurad, K. Shiwaku, Y. Yamane, Screening for antioxidant activity in edible plant products: comparison of low-density Lipoprotein oxidation assay, DPPH radical scavenging assay, and folin-ciocalteu assay, J. Agric. Food Chem. 52 (2004) 2391−2396.

[29] D. Huang, O. Boxin, L. Ronald, Prior the chemistry behind antioxidant capacity assays, J. Agric. Food Chem. 53 (2005) 1841−1856.

[30] M. Adaika, B. Ramdani, Optimisation Des Conditions D'extraction Des Composés Phénoliques Par Ultrason Des Feuilles De *Phoenix Dactylifera* L, Université Echahid Hamma Lakhdar El Oued, Algérie, 2015.

[31] A. Telli, N. Mahboub, S. Boudjeneh, O.E.K. Siboukeur, F. Moulti-Mati, Optimisation des conditions d'extraction des polyphenols de dattes lyophilisees (*Phoenix Dactylifera L*) Variete Ghars, Annales des sciences et technologie 2 (2010) 107−114.

[32] A.M. Gomez-Caravaca, M. Gomez-Romero, D. Arraez-Roman, A. Segura-Carretero, A. Fernandez-Gutierrez, Advances in the analysis of phenolic compounds in products derived from bees, J. Pharm. Biomed. Anal. 41 (2006) 1220−1234.

[33] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventos, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods Enzymol. 299 (1999) 152−178.

[34] K. Tawaha, F.Q. Alali, M. Gharaibeh, M. Mohammad, T. Elelimat, Antioxidant activity and total phenolic content of selected Jordanian plant species, Food Chem. 104 (2007) 1372−1378.

[35] A. Kissoum, K. Khalfaoui, Evaluation phytochimique et étude des activités biologiques d'une plante médicinale Algérienne (*Foeniculum vulgare*), Université des Frères Mentouri Constantine, Algerie, 2015.

[36] M. Kebièche, Z. Lakroun, Z. Mraïhi, R. Soulimani, Anti-diabetogenic and cytoprotective effect of butanolic extract of *Ranunculus repens* L. And quercetin on an experimental model of alloxanic diabetes, Phytotherapy 9 (2011) 274−282.

[37] S. Athamena, Etude quantitative des flavonoides des graines de cuminum cyminum et les feuilles de *rosmarinus officinalis* et l'evaluation de l'activite biologique, Universite el-hadj lakhdar-batnafaculte, Algerie, 2009.

[38] D.O. Kim, C.Y. Lee, Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship, Crit. Rev. Food Sci. Nutr. 44 (2004) 253−273.

[39] S. Bentafar, M. Chaib, Contribution à l'étude phytochimique et à l'effet antioxydantde l'extrait méthanolique *d'Eruca vesicaria*, Mémoire de Master en Sciences de la Nature et de la Vie, Algerie, 2016.

[40] M. Gherib, *Etude des activités antimicrobienne et antioxydante des huiles essentielle et des flavonoides* d'Artemisia herba alba Asso ; Artemisia judaica .L. ssp. Sahariensis; Artemisia campestris L; Herniaria mauritanica Murb et Warionia saharae Benth. *et Cou*, Université Abou Bekr Belkaid-Tlemcen, Algérie, 2009.

[41] Z. Mohammedi, Etude Phytochimique et Activités Biologiques de quelques Plantes médicinales de la Région Nord et Sud Ouest de l'Algérie, Université Abou bekr Belkaid, Algerie, 2013. Thèse en Biologie.

[42] M. Gagandeep, S.B. Kalidhar, Chemical constituents of crataevanurvala (Buch-ham) leaves, Indian J. Pharm. Sci. 68 (2006) 804−806.

[43] H. Falleh, R. Ksouri, K. Chaieb, N. Karray-Bouraoui, N. Trabelsi, M. Boulaaba, C. Abdelly, Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities, C. R. Biologies. 331 (2008) 372−379.

[44] E. Czapecka, A. Mareczek, M. Leja, Antioxidant activity of fresh and dry herbs of some Lamiaceae species, Food Chem. 93 (2005) 223−226.

[45] C. Salih, Etude Phytochimique Et Biologique De Six Plants Medicinales De L'Est De L'Algerie, Université Constantine 1, Algerie, 2013. These En Sciences.

[46] B. Shan, Y.Z. Cai, M. Sun, H. Corke, Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents, Food Chem. 53 (2005) 7749−7759.

[47] A. Matkowski, P. Tasarz, E. Szypuła, Antioxidant activity of herb extracts from five medicinal plants from *Lamiaceae*, subfamily *Lamioideae*, J. Med. Plants Res. 2 (2008) 321−330.

[48] S. Boussahel, Étude biochimique et histologique de l"effet de quelques extraits des plantes toxiques dans la région de Sétif, Université Ferhat Abbes, Sétif, Algérie, 2011. Thèse en Biologie et Physiologie végétale.