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Research article

Total phenolic and flavonoid contents of *Artemisia ifranensis* J. Didier plant extracts and their antioxidant activity

Hanane Elazzouzi [1]*, Nadia Zekri[1], Touriya Zair[2], Mohamed Alaoui El Belghiti [1]

[1]University Mohammed V Faculty of Sciences, Rabat, Morocco
[2]University Moulay Ismail Faculty of Sciences, Meknes, Morocco

ABSTRACT

This study aimed to obtain the proper solvent for extracting bioactive compounds from *Artemisa ifranensis* J. Didier. Moreover, the total phenolic, flavonoid contents, and the antioxidant activities of *Artemisia ifranensis* leaves extracts from Morocco were evaluated. The total phenol and flavonoids contents from various extracts of the vegetal specie were determined by using Folin-Ciocalteu and $AlCl_3$ assays respectively. The results showed that ethyl acetate extract of exhibited the highest phytochemical composition (total phenolic content 35,81 milligrams of gallic acid equivalent/ gram of Extract (mg GAE/g), total flavonoids content 31,85 milligrams of Quercitin/grame of Extract (mg QE/g). Thereafter, the antioxidant activity of extracts was evaluated by the capacity of free radical trapping DPPH• (1,1-Diphenyl-2- picrylhydrazyl). The results reported that the scavenger power of phenolic extracts compared to the standard antioxidant was efficient and showed potential antioxidant properties. Similarly, the best antioxidant capacity was on served in ethyl acetate extract; it has generally the lowest value IC_{50}= 0,656 mg/ml. Data from current results revealed that this plant act as a valuable source of antioxidants.

Keywords: *Artemisia ifranensis* J. Didier, Total phenolic content, Flavonoid content, DPPH, Antioxidant activity.

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Correspondence: Dr. Hanane Elazzouzi* ✉ hananeelazzouzi@yahoo.fr

Department of Chemistry, University Mohammed V Faculty of Sciences, BP. 1014 RP, Rabat, Morocco.

INTRODUCTION

Medicinal plants, as source of remedies, are widely used as alternative therapeutic tools for the prevention or treatment of many diseases. A large number of medicinal plants have been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents is mainly attributed to phenolic compounds such as flavonoids, phenolic acids, tanninsetc. They are very effective to prevent the destructive processes caused by oxidative stress [1].

Natural antioxidants, including herbal remedies [2], have become the target of a great number of research studies in finding the sources of potentially safe, effective and cheap antioxidants.

Artemisia ifranensis J. Didier was selected for this study considering their medicinal properties, belonging to the Asteraceae family or more commonly known as wormwood of Ifrane, endemic and rare from Morocco [3]. It is confined to the Middle-Atlas (Ifrane region, Selrhert plain, daya Chiker (Taza region)) and to the eastern high atlas (Atlas de bni Mellel, Jbel Fernissou) [4]. Thus, this plant has been used in folk medicine for the treatment of gastrointestinal illnesses.

The plant is highly effective against some pathogens thus confirming its use as antirabies [5]. In this investigation, along with phenolic properties of selected plant, antioxidant activities *(in vitro*) of the various extract of plant are also tested. The results could allow the development of this species as an effective therapeutic approach in traditional medicine in Morocco.

MATERIALS AND METHODS

Plant Material

The leaves of *A. ifranensis* were collected, on April, from Timahditeregion in Moroccan Middle Atlas. Afterward, the dried leaves were pulverized and then used for preparation of various extracts. The botanical identification of the samples was confirmed by Professor Mohamed Ibn Tattou, National Herbarium of Rabat Scientific Institute (Morocco).

Extraction of Phenolic Compound

A mass of 30 g of the crushed dry leaves of *A. ifranensis* was macerated to 300 ml of methanol (70%) at room temperature every 48 hours. The filtered solution was evaporated at reduced pressure (40°c) and then fractionated, according to the Bruneton's (1993) slightly modified method [6]. The aqueous phase was subjected

to successive extractions liquid-liquid (fractionation) and has been manipulated using organic solvents (ethyl acetate and *n*-butanol).

Dosage of Total Phenols of Plant Extracts

The total phenol content in the extracts was determined by the modified Folin-Ciocalteu method [7]. 200 µl of extract sample of known concentration was mixed with 1,5 ml Folin-Ciocalteu reagent, previously diluted of 10%, and 1,5 ml (7,5 % (w/v)) of aqueous sodium carbonate solution. The reaction mixture was homogenized and get arrested for 2 hours at room temperature for color development. The absorbance reading of each solution was determined at 765 nm with a Shimadzu UV-MINI 1240 spectrophotometer. The results wers expressed in milligrams of gallic acid equivalent per gram of dry matter (mg GAE/g of dry plant), using the equation based on the calibration curve of gallic acid: y = 0,095x + 0,003

Dosage of Flavonoids in Plant Extracts

The flavonoids were quantified by the direct dosing with aluminum trichloride using the method of Djeridane et al., (2006) [8]. A volume of 100 µl of sample solution was added with 100 µl of 10% (m/v) aluminum trichloride ($AlCl_3$). After 30 min at room temperature, the absorbance was determined at 433 nm using the spectrophotometer cited above. The flavonoid content was expressed in milligrams of quercetin equivalent per gram of dry matter (mg QE/g of dry plant) and was calculated from the equation of calibration curve with quercetin standard: y = 0,073 x – 0,081.

Evaluation of Antioxidant Activity By DPPH˙ Free Radical Scavenging Method

The procedure reported by Nikhat et al., (2009) was adopted to determine the scavenging ability of DPPH [9]. A volume of 200 µl of various concentrations of diluted extracts, prepared in ethanol at a rate of 1,6 mg/ml, was added to 2,8 ml of DPPH at $6{,}10^{-5}$ M. Ascorbic acid has been used as a positive control. All solutions were mixed and stored in the dark for half hour, and finally, the absorbance was taken at 517 nm. DPPH scavenging ability of plant extracts was expressed as the percentage of reduction or inhibition (I %) of free radical DPPH˙ [10].

The graph of the absorbance variation according to the concentration of extract allowed determining the IC_{50} (extract concentration providing 50 % inhibition). The values of IC_{50} were obtained from the 3rd degree polynomial trend curves [11].

Statistical Analysis

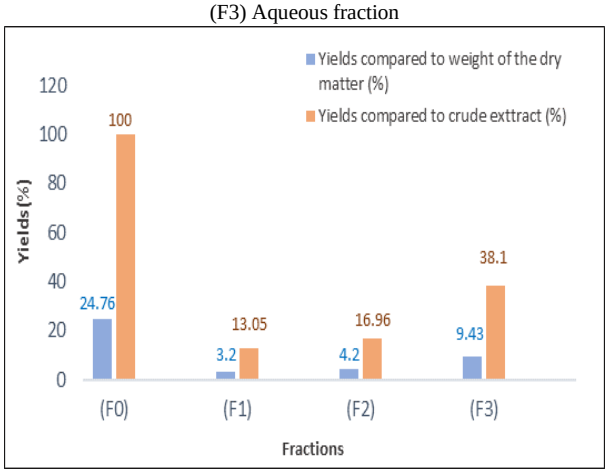
The experimental data obtained were expressed as an average. The statistical analysis of the data, including the correlation coefficient of the antioxidant properties, was carried out using Excel software (Microsoft Office 2010).

RESULTS AND DISCUSSIONS

Yield of Phenolic Extracts

The yields of different extracts are given in Figure 1. The interesting yields of the various extracts from *A. ifranaens* is were reported, and that hydro methanolic represented the highest yield in *A. ifranensis* (24,76 %). Thus, the aquoeous, butanolic and Ethyl acetate extracts of *A. ifranens* is recorded contents in extractable compounds of 9,43 %, 4,2 %, and 3,2 % respectively. They are the significant losses of material given the relatively high number of washes carried out during fractionation of the crude extract. In fact, the yields decreased in the decreasing direction of the solvent's polarity used.

Figure 1: Yields of different leaves extracts of *Artemisia ifranensis* (F0) Crude extract; (F1) Ethyl acetate Fraction; (F2) *n*-Butanol fraction; (F3) Aqueous fraction



Data processing of the obtained results showed that total phenolic and flavonoid compounds varied greatly among different solvents, this indicated the possible influence of extracting solvent on total phenolic and flavonoid contents.

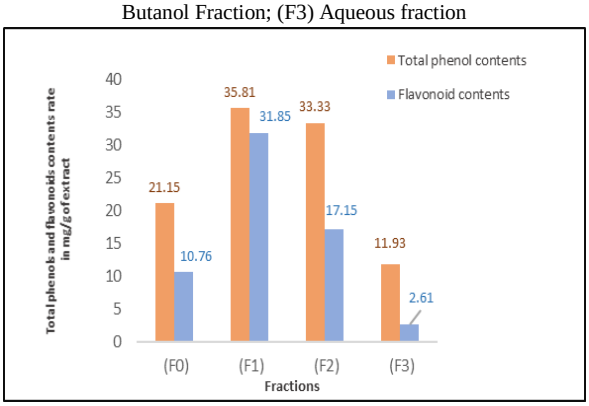
In fact, it is important to compare our results with those of other similar ones on *Artemisia* sp., from Morocco. Amine et al., (2018) reported interesting yields which remains less important of our found results and differs according to the solvents polarity [12].

Hence, we can deduce that polar solvents give better yields than the apolarones; it is important to emphasize that the polarity of the solvent affects the extraction content; and made it possible to separate the different metabolites found according to their degree of solubility in the extraction solvent, and according to their structural complexity [13].

Quantitative Analysis of Total Phenols and Flavonoids of Plant Extracts

The results presented in figure 2 shows that the total phenols and flavonoid contents vary considerably between the different extracts. This observation is confirmed by the low values recorded in the residual aqueous fraction. Indeed, according to the results, all *A. ifranensis* extracts are richer in total phenols (21,15; 35,81; 33,33; 11,93 mg GAE/g Extract respectively) than in flavonoids (10,76; 31,85; 17,15; 2,61 mg QE/g Extract). Similar results have been reported by Boudiaf (2006) [14]. However, different results have been reported in the work of Talbiet al., (2015), and Elazzouziet al., (2018) [15, 16].

Figure 2: Quantity of phenolic compounds and flavonoids from *Artemisia ifranens* is extracts (F0) Crude extract; (F1) Ethyl acetate Fraction; (F2) *n*-Butanol Fraction; (F3) Aqueous fraction



Dosage of Total Phenols

The measures of the colorimetric analysis are summarizes as well in figure2. A variation in total phenolic contents of *A. ifranens* is leaves was observed. Ethyl acetate extracts generally present the highest levels of polyphenols (35,81 mg GAE/g Extract), followed by *n*-butanolic fraction (33,33 mg GAE/g Extract), crude extract (21,15 mg GAE/g Extract) and the residual aqueous fraction (11,93 mg GAE/g Extract).

These results are in agreement with that observed by Lee et al., (2013) who found that ethyl acetate extract of *A. absinthium* from Algeria is richer in polyphenols than the aqueous extract [17]. Also, results have been described by Laouini et al., (2016) reveal that ethyl acetate and butanolic extracts of *A. herba alba* have been significant contents of polyphenols (75,64 ± 2,97 and 92,29 ± 3,25 mg GAE/g dry weight respectively) [18]. Through this quantitative method, other authors find discordant results. It appears that the methanolic extract contained the highest amount, followed by the ethyl acetate extract in work of Ahameethunisa et al., (2012) [19]. Likewise, another assay test reported that polyphenol contents in the polar (water) is higher than a polar (chloroform) solvents of *A. frangrans* [20].

Dosage of Flavonoids

The results of the colorimetric analysis are given in figure 2. The flavonoids contents (FCs) of extracts varied between 2,61and 31,85 mg QE/g Extract. The highest levels of flavonoids were observed generally in ethyl acetate fraction (31,85 mg QE/g Extract), followed by *n*-butanolic fraction (17,15 mg QE/g Extract). However, the Aqueous extract has recorded the lowest content (2,61 mg QE/g Extra it). In a survey and in comparison, of past literature, the results of the present study of *A. ifranens* is, are clearly interesting compred to the result of *A. Absinthium* dosing extracts [17]. Similarly, the dosing of ethyl acetate extract of *A. herba alba* from Algeria showed hight level of flavonoids (76,55 mg QE/g dry weight) [21]. Indeed, Laouini et *al*. (2016) reported that ethyl acetate extract has a flavonoid content than butanolic extract of *A. herbaalba* from Algeria [18]. In fact, other study has obtained flavonoid quantification results better than those of our work [22].

Hence, it appears that the flavonoid contents of ethyl acetate extract of *A. compestris* from Algeria was lowest and in discordance with our results [23].

Evenly, variability of phenolic content is probably due to the genotopic and environmental factors (namely climate, location, temperature, fertility, diseases, pest exposure, and also the degree of plant maturity and storage duration). In addition, the phenols compounds dosing is conditioned by several other factors in cluging the extraction method, the standard and the quantification method [24].

The different phases do not have the same richness in these compounds, which suggests that the difference in solubility of the phenolic compounds (including flavonoids) is due to the polarity of the chosen solvents. Also, the chemical nature, the degree of polymerization and the interaction of these compounds with the other constituents have a strong influence on the quantification of polyphenols and flavonoids [25].

Total Phenolic Content measured by the Folin-Ciocalteu procedure may not be able to give a complete image of the quality or quantity of the phenolic constituents in the extracts [26]. Despite its high sensitivity, this method may present interference problems. Indeed, the Folin-Ciocalteureagent can react with amino-acids and reducing sugars such as glucose and fructose [24].

Antioxidant activity of *A. ifranensis* Extracts

The antioxidant activity of the extracts was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) test system. Figure 3 demonstrates DPPH scavenging activity, expressed in percentage, caused by different concentrations of solvent extracts from *A. ifranens* is the antioxidant power was characterized also by the parameter IC_{50}. The lower IC_{50} value corresponds to the higher antioxidant activity.

In fact, according to the results present in figure 4, extracts of *A. ifranens* is were able to reduce the free radical DPPH· Compared to the standard (IC_{50} of ascorbic acid =0,051 mg/ ml). Ethyl acetate phase is the most effective, IC_{50}= 0,656 mg/ml, by comparison to the hydro methanolic and *n*-butanolic extracts.

Moreover, results have been described in previous publications established on the antioxidant activities. Thus, in the species of *A. herba alba* from Algeria, in 2010, Khennouf et al., reported that the ethyl acetate is the active extract, and reduce free radicals to IC_{50} values of 32,9 ± 0,036 µg / ml, an IC_{50} reference (BHT) is of 17,8 ± 0,022 µg / ml [21]. In agreement with our results revealed, ethyl acetate, methanolic (50%), and butanolic extracts for *A. macrocephala* from Pakistan are endowed with an important antiradical power successively [27]. Indeed, Lee et al., (2013) reported different results to ours in their work. The methanolic extract had a hight antioxidant activity following by ethyl acetate extract of Korean

A. absinthium [17]. Moreover, many researchers have also reported an antiradical efficacy of *Artemisia sp.* (*A. fragrans*, and *A. argyi*) [20,22].

Figure 3: Percentage inhibition of the free radical DPPH• as a function of different concentrations used extracts of *Artemisia ifranens* is (crude (a), ethyl acetate (b), *n*-butanolic (c)), and that of ascorbic acid (d)

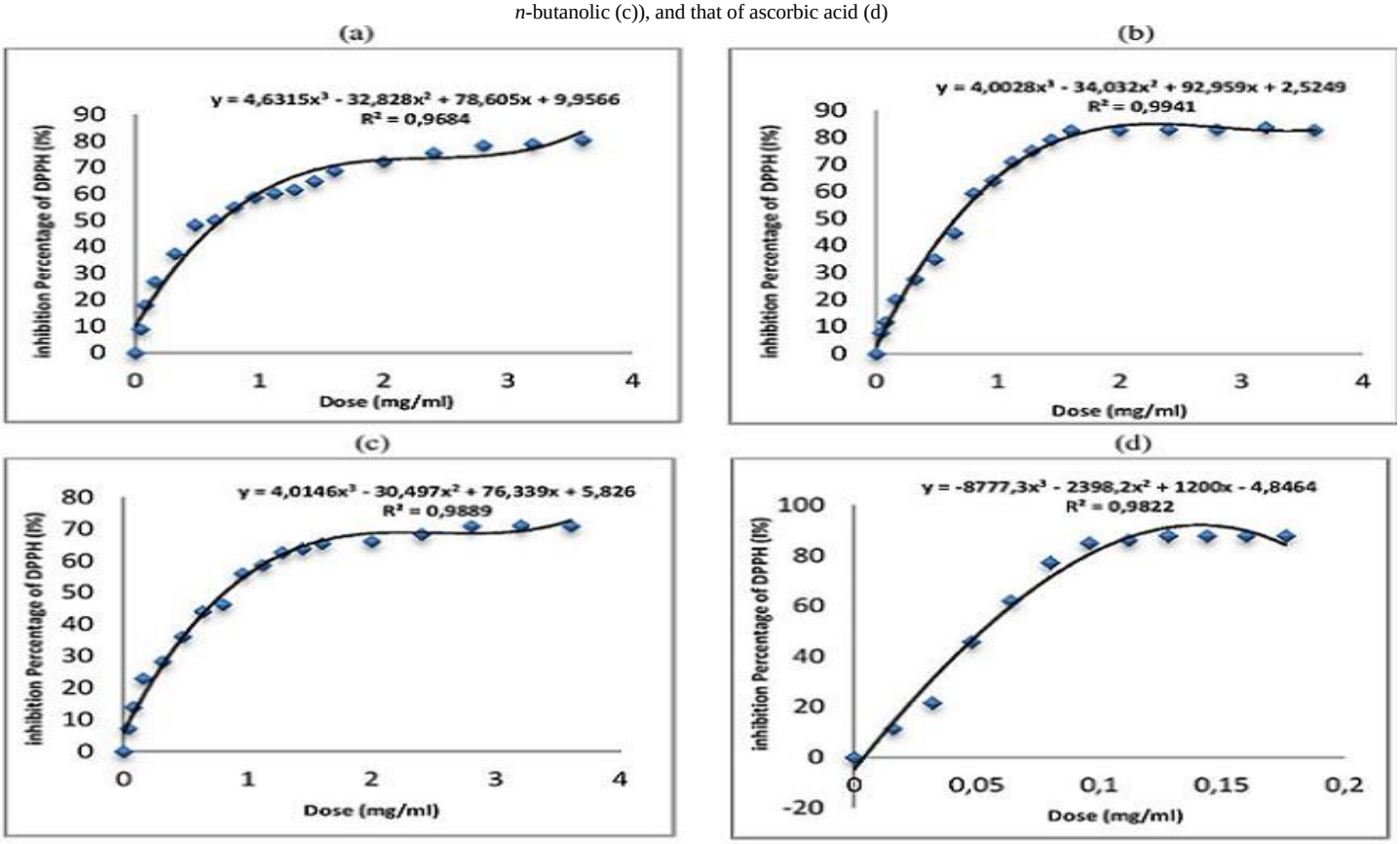
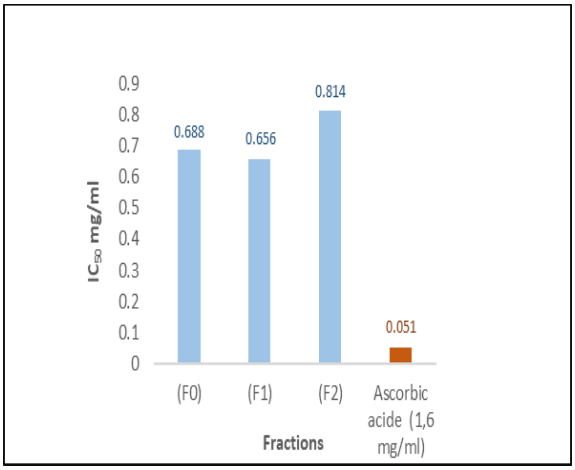


Figure 4: IC_{50} values Illustration of different extracts of *Artemisia ifranensis*, and Ascorbic acid



In this study, by combining the results, we have been able to demonstrate the existence of a close relationship, heterogeneity in the correlation analysis that linked IC50 valuesand the total phenol or flavonoid contents. Indeed, in view of the results, a positive correlation has been observed in ethyl acetate extract of the specie studied, which is the richest in phenolic compounds and therefore has hight antioxidant activity. However, there is one exception to report. These are the crude and butanolic extracts which have distinct contents in total phenols and flavonoids contents; it appears that the extract that possess the highest phenolic compound content, it has shown its inferiority in terms of antioxidant activity.

Indeed, generally, the antioxidant capacity is strongly dependent on the concentration in phenolic compounds. However, in the case of our results this relationship is not always obvious since it can be insignificant in some cases. Indeed, Djeridane et al., (2006) estimate that the existence of a synergy between the various phenolic compounds can be determining in the antioxidant capacity of a given plant. Thus, this activity does not depend only on the basis of their phenolic content (quantitative) but also required their proper qualitative characterization (chemical structure and the interaction between the various compounds) [8].

CONCLUSION

The replacement of synthetic with natural antioxidants, because of implications for human health, may be advantageous. The present study reported the antioxidant activity, quantitative analysis of total phenolic and flavonoid contents of an endemic Moroccan species: *Artemisia ifranensis*. In order to realize the health benefits from potential plant sources, it is important to measure the antioxidant activity using radicals and oxidation systems. *In vitro*, The ethyl acetate extract exhibited highest toalphenolic and flavonoids content (35,81 mg GAE/g Extract and 31,85 mg QE/g Extract respectively) and possess highest antioxidant activity (IC_{50} value 0,656 mg/ml) compared to crude and butanolic extracts. Heterogeneity in the correlation analysis has been observed, it is widely accepted that this is not necessarily the high content of

phenolic compounds exhibits a powerful antioxidant activity, but also the chemical structure of the antioxidant compounds.

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DECLARATIONS

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Conflict of interest

The authors declare no conflict of interest.

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