

## Evaluation of the Antioxidant Potential of Some Morocco Medicinal Plants

<sup>1,2,3</sup>Mina Moussaid, <sup>2</sup>Abdel aziz Elamrani, <sup>3</sup>Chady Berahal, <sup>3</sup>Hassane Moussaid,  
<sup>1</sup>Nourdinne Bourhime and <sup>2</sup>Mouhamed Benaissa

<sup>1</sup>Department of Biology, Laboratory of Biochemistry, Cellular and Molecular Biology, Faculty of Science I, Aïn Chock, University Hassan II, BP 5366, Casablanca Maarif, 20100. Morocco

<sup>2</sup>Department of Chemistry, Laboratory of Synthesis, Extraction and physic-chemical study of organic molecules, "Team Food Chemistry of Natural Substances and Marines," I Faculty of Science, Aïn Chock, University Hassan II. B 5366 P, Maarif, Casablanca, 20100. Morocco

<sup>3</sup>Laboratory of Pharmaceutical Chemistry, Institute of Pharmacy, Campus de la Plaines, Boulevard du Triumphant, 1050 Brussels Belgium

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**Abstract:** Medicinal plants are a source for a wide variety of natural antioxidants. In the study reported here, we have conducted a comparative study between five medicinal plants having the same geographic origin: the Casablanca region in the West of Morocco and growing in the same natural conditions. The amount of total phenolics and total flavonoids from different parts of plants used in Moroccan popular medicine were evaluated. Furthermore, antioxidant activities for these parts using vitamin C equivalent antioxidant capacity (VCEAC) test were also evaluated. The results show that the antioxidant activities varied greatly among the different plant parts used in this study and some plants are rich in natural antioxidants especially leaves of *Mentha pulegium (L.)* and of *Marrubium vulgare (L.)*. A positive correlation between total phenolic or flavonoid contents and VCEAC was found with a correlation coefficient of  $R^2 = 0.939$  and  $R^2 = 0.837$ , respectively. These findings show that phenolics in these plants provide substantial antioxidant activity.

**Key words:** Medicinal Plants • Phenolics • Flavonoids • Vitamin C Equivalent Antioxidant Capacity (VCEAC)

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### INTRODUCTION

Oxidative stress is mediated by reactive oxygen species (ROS) which are generated during the normal and aberrant cellular metabolism that utilizes molecular oxygen. The imbalance between production of ROS like  $O^{\cdot-}$ ,  $H_2O_2$ ,  $OH^{\cdot}$ ,  $ROO^{\cdot}$  and the capacity of the normal detoxification systems in favor of the oxidants leads to oxidative stress, which itself leads to cellular damage caused by the interaction of ROS with cellular constituents. Oxidative stress is involved in many acute and chronic diseases including cancer, cardiovascular troubles and neurodegenerative diseases. The balance between antioxidation and oxidation is believed to be critical in maintaining a healthy biological system [1- 3].

Recently, many researchers have taken a great interest in medicinal plants for their phenolic

concentrations and related total antioxidant potential [2, 4, 5]. It is reported that some medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than dietary plants [5]. Many investigations indicate that these compounds are of great value in preventing the onset and/or progression of many human diseases [6].

The health-promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting reactive oxygen species (ROS) [5]. The purposes of this study were to determine the content of total phenolics and total flavonoids and to evaluate total antioxidant activity of five Moroccan medicinal plants using the vitamin C equivalent antioxidant capacity (VCEAC) test.

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**Corresponding Author:** Mina Moussaid, Department of Biology, Laboratory of Biochemistry, Cellular and Molecular Biology, Faculty of Science I, Aïn Chock, University Hassan II, BP 5366, Casablanca Maarif, 20100. Morocco,

## MATERIALS AND METHODS

**Chemicals:** Aluminium chloride ( $\text{AlCl}_3$ ), chlorogenic and Gallic acid were purchased from Sigma Chemical Company. Ascorbic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), PBS buffer, 2,2'-azobis(2-methylpropionamide) dichloride (AAPH), Folin-Ciocalteu's phenol reagent and Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) were purchased from Acros Organics.

**Plant Material and Protocol:** Five medicinal plants were collected from the Casablanca region in the west of Morocco. Plant parts have been chosen in relation to Moroccan popular medicine use: Leaves and flowers of two plants: *Mentha pulegium* (L.) (Lamiaceae) and *Origanum majorana* (L.) (Lamiaceae); leaves and flowering tops of *Marrubium vulgare* (L.) (Lamiaceae), tuberous root of *Asphodelus cerasiferus* Gay (Liliaceae) and bulbs of *Allium subvillosum* (L.) (Liliaceae), these plants are spontaneous and perennials flora of the Casablanca region. The extractions were carried out using the same protocol. Plant parts air dried, grinded and macerated in pure water for 12 hrs at room temperature and then for 12 hrs at 37°C temperature. Afterwards the filtrate was concentrated.

**Determination of Total Phenolics:** Total phenolic contents were evaluated with Folin-Ciocalteu phenol reagent [7] using spectrophotometric analysis (Perkin-Elmer Lambda 40 UV/VIS spectrophotometer). Briefly, an aliquot (1 ml) of standard solutions of chlorogenic acid at different concentrations or appropriately diluted extracts was added to a 25 ml volumetric flask containing 9 ml of  $\text{H}_2\text{O}$ . A reagent blank using  $\text{H}_2\text{O}$  was prepared. One milliliter of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7%  $\text{Na}_2\text{CO}_3$  solution was added with mixing. The solution was then immediately diluted to volume (25 ml) with  $\text{H}_2\text{O}$  and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was read at 750 nm. Total phenolic contents in medicinal plants were expressed as mg chlorogenic acid equivalents (CAE)/g dry weight. Samples were analyzed in three replications.

**Determination of Total Flavonoid:** Total flavonoid contents were measured according to a colorimetric assay,

based on the formation of a complex flavonoid-aluminum, having an absorptivity maximum at 430 nm. All determinations were made in triplicate and values were calculated from a calibration curve obtained with quercetin. Total flavonoid contents in medicinal plants were expressed as mg quercetin equivalents (qE)/g dry weight (dw) [8].

**Determination of Total Antioxidant Activity Using Abts Radical Scavenging Capacity Assay:** VCEAC test developed by Kim *et al.* [7] was used in this study. Total antioxidant activities of medicinal plants were determined by scavenging blue-green ABTS radicals and were expressed as mg vitamin C equivalent (VCE) per g dry weight. Briefly, 1 mM AAPH, a radical initiator, was mixed with 2.5 mM ABTS in phosphate-buffered saline (PBS, pH 7.4). The mixed solution was heated in a water bath at 68°C for 13 min. The resulting blue-green ABTS radical solution was adjusted to the absorbance of  $0.650 \pm 0.020$  at 734 nm with additional PBS. Twenty microliters of sample was added to 980  $\mu\text{l}$  of the ABTS radical solution. The mixture was incubated in a 37°C water bath under restricted light for 10 min. The control consisted of 20  $\mu\text{l}$  50% methanol and 980  $\mu\text{l}$  of ABTS radical solution. The decrease of absorbance at 734 nm was measured 10 min later. Samples were analyzed in three replications.

**Statistical Analysis:** Data were reported as mean standard deviation. To examine antioxidant activity differences between extracts, we have used ANOVA followed by PLSD of Fisher test. For all statistical comparisons, the level of significance was set at  $p < 0.05$ . All statistical analyses were carried out using the Statview 4.5 statistical package (Abacus Concepts, Inc.).

## RESULTS

**Determination of Total Phenolics:** Table 1 show the traditional uses of some plants in Moroccan society and their total phenolic contents, which varied between 6.7 and 33.9 mg of CAE/g (dw). The highest concentration of total phenolics was observed in leaves and flowers of *M. pulegium*, followed by leaves and flowers of *O. majorana*, leaves and flowering tops of *M. vulgare* and bulbs of *A. subvillosum*. The root of *A. cerasiferus* had the lowest phenolics concentration.

Table 1: Medicinal uses of some Moroccan plant parts and their amounts of total phenolics

Scientific name	Plant parts used in Moroccan popular medicine and their effects and/or uses	Total phenolics (mg CAE/g dw)
<i>Allium subvillosum</i> (L.)	Bulbs: micturition disorders, mumps, tinea	11.2±1.31
<i>Asphodelus cerasiferus</i> Gay	Tuberous root: treatment of ear infections, care of abscesses	6.7±1.19
<i>Marrubium vulgare</i> (L.)	Leaves and flowering tops: bronchitis, diabetes	24.2±2.59
<i>Mentha pulegium</i> (L)	Leaves and flowers: bronchitis, spasms	33.9±2.14
<i>Origanum majorana</i> (L.)	Leaves and flowers: inflammation, cough	26.5±1.21

The data are displayed with mean ± standard deviation of three replications. The contents of total phenolics in plants were expressed as chlorogenic acid equivalent (CAE) per 1 g dry weight.

Table 2: Total flavonoid contents in parts of some Moroccan medicinal plants and their total antioxidant activities quantified by VCEAC assay.

Scientific name	Total flavonoids (mg qE/g dw)*	VCEAC (mg VCE/g dw)*
<i>A. subvillosum</i>	8.76±0.004	14.4±0.05
<i>A. cerasiferus</i>	4.25± 0.02	7.38±0.45
<i>M. vulgare</i>	13.18 ±0.03	19.96 ±0.42
<i>M. pulegium</i>	16.75±0.11	20.12±0.25
<i>O. majorana</i>	11.0 ± 0.32	16±0.21

\* The data are displayed with mean ± standard deviation of three replications. The contents of total flavonoids in plant parts and their total antioxidant activities estimated by VCEAC assay were expressed as quercetin equivalent and vitamin C equivalent (VCE) per 1 g dry weight, respectively.

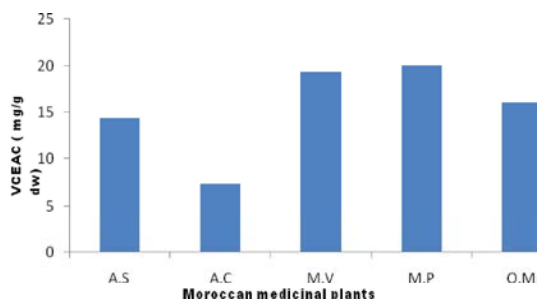


Fig. 1: Comparison of total antioxidant activities for parts of some Moroccan medicinal plants estimated by VCEAC assay and expressed as vitamin C equivalent (VCE) per g dry weight. M.P, M.V, O.M, A.S, A.C, Stand for Leaves and flowers of *M. pulegium*, leaves and flowering tops of *M. vulgare*, leaves and flowers of *O. majorana*, bulbs of *A. subvillosum* and root of *A. cerasiferus*, respectively. Data represent mean ± standard deviation of three replications. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Determination of Total Flavonoids:** The concentrations of total flavonoids of five Moroccan medicinal plants varied between 4.25 and 16.75mg of CE/ g dw (Table 2). Leaves and flowers of *M. pulegium* exhibited the highest flavonoids concentration followed by leaves and flowering tops of *M. vulgare*, leaves and flowers of *O. majorana* and bulbs of *A. subvillosum*. The root of *A. cerasiferus* had the lowest flavonoids concentration.

**Antioxidant Activities:** The total antioxidant activities quantified by VCEAC assay are presented in Table 2. ANOVA revealed significant differences between extracts (Fig. 1) with respect to total antioxidant activity. Fisher test did not reveal any significant difference between Leaves and flowers of *M. pulegium* and leaves and flowering tops of *M. vulgare* with respect to total antioxidant activity ( $p > 0.05$ ). Leaves and flowers of *O. majorana*, bulbs of *A. subvillosum* and root of *A. cerasiferus* exhibited significantly less antioxidant activity than Leaves and flowers of *M. pulegium* and leaves and flowering tops of *M. vulgare* ( $p < 0.01$ ;  $p < 0.001$ ;  $p < 0.001$ , respectively).

## DISCUSSION

The total phenolic and the total flavonoid contents of 1 g dry weight of plant parts traditionally used ranged from 6.7 to 33.9 mg of CAE and from 8.76 to 16.75 mg of qE, respectively (Tables 1 and 2). The total phenolics and the total flavonoids showed the similar tendency in ranking: Leaves and flowers of *M. pulegium* by leaves and flowering tops of *M. vulgare*, leaves and flowers of *O. majorana*, bulbs of *A. subvillosum* and root of *A. cerasiferus*. The three first medicinal plant parts are potentially rich sources of natural antioxidants, in fact, these plants are of the same family. However, root of *A. cerasiferus* are poor in polyphenols, their total phenolic and flavonoid amounts were approximately five fold and four-fold lower than Leaves and flowers of *M. pulegium*, respectively.

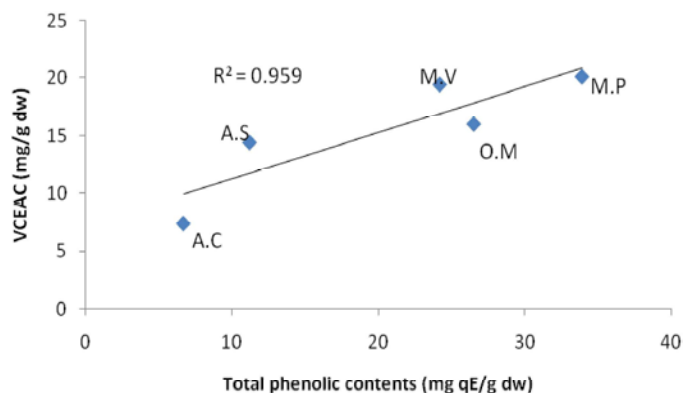


Fig. 2: Positive correlation between total phenolics and VCEAC for some Moroccan medicinal plants, M.P, M.V, O.M, A.S, A.C, Stand for Leaves and flowers of *M. pulegium*, leaves and flowering tops of *M. vulgare*, leaves and flowers of *O. majorana*, bulbs of *A. subvillosum* and root of *A. cerasiferus*, respectively.

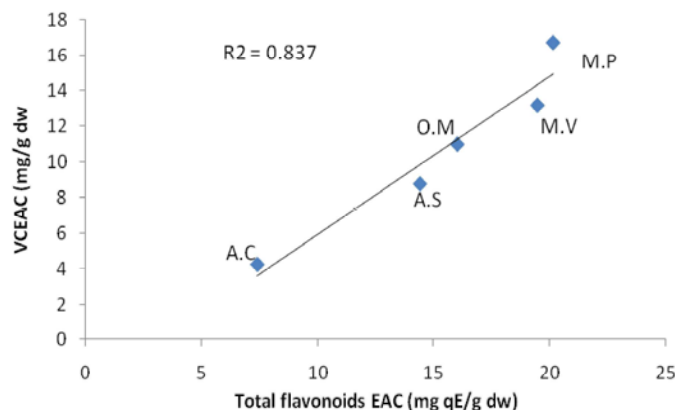


Fig. 3: Positive correlation between total flavonoids and VCEAC for some Moroccan medicinal plants: M.P, M.V, O.M, A.S, A.C, Stand for Leaves and flowers of *M. pulegium*, leaves and flowering tops of *M. vulgare*, leaves and flowers of *O. majorana*, bulbs of *A. subvillosum* and root of *A. cerasiferus*, respectively.

The medicinal plants have the same geographic origin and grow in the same natural conditions, nevertheless the plants belong to different families and the parts used in this study are not the same. It is well known that the amount of total phenolics vary in different parts of the same plant, moreover it has been reported that the amount of total phenolics vary with respect to families and varieties [4, 9, 10].

To evaluate antioxidant activities VCEAC test developed by Kim *et al* [7] was employed. This test is a good method for measuring the antioxidant activity of extracts or individual chemical compounds [7, 11]. The different plant parts used display scavenging activities for ABTS radical. We found that the total antioxidant activities varied greatly among the different parts, since they ranged from 7.38 to 20.12 mg of VCE/g dw (Table 2).

Based on these data, we can classify medicinal plants into two groups. The first one showing an identical and high antioxidant activity profile constituted by Leaves and flowers of *M. pulegium* and of *O. majorana*, the leaves and flowering tops of *M. vulgare*. The second one exhibiting low antioxidant activity constituted by bulbs of *A. subvillosum* and root of *A. cerasiferus*. The difference between these two groups was significant with respect to antioxidant activity (Fig. 1).

Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions [12]. Phenolic constituents, such as flavonoids, phenolic acids and tannins are well known for their high antioxidant activity [13, 14].

Epidemiological studies suggest that the consumption of flavonoid-rich foods protects against

human diseases associated with oxidative stress, like coronary heart disease and cancer [15, 16]. The protective effect provided by fruits and vegetables against cancer, cardio and cerebrovascular diseases, has been attributed to their antioxidant compounds [17]. The majority of antioxidant capacity of plants is not only represented by vitamin C, vitamin E or  $\beta$ -carotene, but is also due to other compounds such as polyphenols which have a strong antioxidant potential [18]. Many studies indicate a linear relationship between total phenolics and antioxidant activity [4, 7]. In this study, we found that phenolic compounds are major contributors to antioxidant activity, since total phenolics and antioxidant activity showed a good correlation good correlation ( $R^2 = 0.939$ ) (Fig. 2).

However, antioxidant capacity and total flavonoids showed a relatively weak relationship with a correlation coefficient of  $R^2 = 0.837$  (Fig. 3). Our results are in agreement with previous reports that the phenolic compounds contribute significantly to the antioxidant activity in medicinal plants [4, 5, 19].

In conclusion, the amount of phenolics, flavonoids and related total antioxidant activity of some Moroccan medicinal plant parts were evaluated. Antioxidant activity varied greatly among the different plant parts used in this study, but it was highly correlated with the content of polyphenolics.

Therefore, we take an interest in Leaves and flowers of *M. pulegium* and leaves and flowering tops of *M. vulgare*, since they exhibited important antioxidant activities and present a good source of natural antioxidants. After this comparative study, our objective will be identification and determination of the amount of individual polyphenolics responsible for the majority of antioxidant activity in parts of these two plants.

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