International Journal of New Technology and Research (IJNTR)
ISSN: 2454-4116, Volume-1, Issue-3, July 2015 Pages 07-11

Antioxidant Activity and Total Phenolic and Flavonoid Contents of 30 Medicinal and Aromatic Plants Located in the South of Morocco

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***Abstract*— In this study, antioxidant activity, total phenolic and total flavonoid contents of 30 species extracts, from 15 botanical families grown on three localities (Agdz, Tafraout and Taroudannt) of Souss Massa Draa (Southern Morocco), were determined using spectrophotometric methods. DPPH radical cation assay was used for evaluation of free radical scavenging properties of investigated species. The results showed a remarkable high antioxidant activity in the majority of tested plants, especially in Tetraclinis articulata, Thymus leptobotrys, and Lavandula stoechas, which neutralized up to 90 % of DPPH radicals. However, Conyza canadensis showed the lowest antioxidant value (88.19%). The total phenolics content measured by Folin-Ciocalteu method, ranged from 2.54 to 55.62 µg of gallic acid equivalents (GAE)/mg of dry matter (DM). Ceratonia siliqua (55.63 µg GAE /mg), Cistus villosus (41.10 µg GAE /mg), Limoniastrum feei (36.52 µg GAE /mg) and Rubus ulmifolius (35.02 µg GAE /mg) had very high levels of phenolics, whereas in Conyza canadensis (2.54 µg GAE /mg) phenolics were quit low. For total flavonoids content, two methods were used. Spectrophotmoetric method using NEU reagent showed a significant levels of flavonoids in Inula viscosa (70.08 µg/mg), Globularia alypum (66.28 µg/mg), Teucrium chamaedrys (63.95 µg/mg), Ruta montana (59.9 µg/mg) and Ononis natrix (55.49 µg/mg). For the second method with aluminium trichloride (AlCl3), the amounts of total flavonoid expressed as rutin equivalents/g DM, were higher in Rubus ulmifolius (117.79 µg/mg), Ononis natrix (80.84 µg/mg), Rhus pentaphulla (67.67 µg/mg), Thymus leptobotrys (53.17 µg/mg) and Thymus satureioides (52.34 µg/mg). According to this method Mentha pulegium exhibited the lowest flavonoids concentration (9.46 µg/mg), while the first method revealed low levels of flavonoids content in Conyza canadensis (5.41 µg/mg).**

***Index Terms*— Antioxidant activity, flavonoids contents, medicinal and aromatic plants, phenolics contents.**

I. INTRODUCTION

Due to its geographical position, Morocco has interesting potentialities in the field of medicinal and aromatic plants, and is classified among the most floristically rich countries in the Mediterranean region [1]. Indeed, the Moroccan flora is diverse with about 4,200 species and subspecies, of which 850 are currently used in traditional medicine [2]. The valley of the Souss-Massa-Draa, located in the south of Morocco, is one of the richest regions in Morocco in terms of plant diversity [3]. This diverse flora is a natural reservoir of bioactive molecules. These natural substances from plants have multiple interests in the food, pharmaceutical and cosmetic industries. Phenolic compounds, particularly flavonoids which are present in all vascular plants, are secondary metabolites used in chemiotaxonomy of the main plant groups [4]. They are also involved in many physiological processes in plants; they are stress biomarkers ensuring the survival of plants under different environmental conditions [5, 6, 4]. In addition several studies have demonstrated the undeniable role of flavonoids in plant protection [7, 8]. Furthermore, the phenolic compounds are natural antioxidants that have attracted special interest because of their involvement in the prevention and treatment of several diseases. In fact, these secondary metabolites have important biological and pharmacoligical activities, such anti-oxidative, anti-inflammatory, antitumoral (antimutagenic) and anticarcinogenic [9, 10, 11, 12].

Today, phenolic compounds especially flavonoids know a new interest which is the search for novel molecules used (usable) in phytotherapy and cosmetics. These substances have hydroxyl groups and their antioxidant properties are attributed in part to their ability to scavenge free radicals [13, 14].

The present study is a part of enhancement of natural plant resources located in the Souss-Massa-Draa region and its basic aim is to quantify phenolic compounds and to evaluate the antioxidant activity of 30 plant species collected from three different locations in southern Morocco and which are used by local population as medicinal and flavouring plants.

II. MATERIALS AND METHODS

A. Plant materials and studied stations

Thirty species belonging to 15 botanical families of medicinals and aromatics plants were collected from three regions of southern Morocco:

- Tafraoute: located in the Anti-Atlas mountains, at 120 Km Southeast of Agadir's town and at an altitude of 1200 m.

- Agdz: Continental locality of the Anti-Atlas, situated at 65 km south of Ouarzazate, at the entrance of the Draa Valley and at a hight of 1100-1400 m.

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- Taroudannt*:* located on the high-Atlas mountains at 80 Km in the east of Agadir. Its altitude is about 2500 to 3000 m.

All the investigated species were botanically characterized (table. 1). Asteraceae, Lamiaceae and Fabaceae were the most representative families with 7, 7 and 4 tested species respectively. Plant materials were collected between March and April. Collected plants parts were different following the species (table. 1). Fresh plant samples were cleaned and air-dried in darkness at room temperature. Dried plant parts were then powdered and stored in the dark at a dry place until further use.

B. Extraction

Each grounded dry plant material (50 mg) was weighed into Eppendorff tube and 1ml of methanol : H2O (80 : 20; v/v) mixture was added. Tubes were then sonicated 20 min and centrifuged at 10000 rpm for 15 min. The obtained extracts were used for antioxidant activity, total phenols and flavonoids determination.

C. Determination of total phenolic contents

The concentration of phenolics in plant extracts was determined by the spectrophotometric method. In brief, a 25 µl of plant extract was mixed with 110 µl of 10 % Folin-Ciocalteu's reagent. After shaking for 3 min, 200 µl of a 5% Na_2CO_3 solution was added to the mixture followed by the addition of 1.9 ml of distilled water and mixed thoroughly. The mixture was thereafter incubated in the dark at 60 °C for 30 min. The absorbance was measured at 725 nm using an IC 6400 visible spectrophotometer [15, 16].

The same procedure was repeated for standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read from calibration line, then the content of phenolics in extracts was expressed in terms of µg gallic acid equivalent (GAE)/ mg of dry weight.

D. Determination of total flavonoids concentration

Quantification of total flavonoids was done with an IC 6400 visible spectrophotometer using two different methods. In the first assay, the flavonoids concentration was determined by method of [17] and [18]. A volume of 2 ml of extract is mixed with 100 µl of Neu's reagent (1% methanolic solution of diphenylboric acid-2-aminoethylester, [19]). The sample absorbance was determined at 404 nm and compared to that of standard quercetin (0.05 mg/ml) treated with the same reagent. The total amount of flavonoids was calculated using the following formula:

$$F = \frac{Aext \times 0.05 \times 100}{Aq \times Cext}$$

Aext : extract absorbance
Aq : (Quercetin (0.05 mg/ml) absorbance)
Cext : Concentration of extracted plant material (mg/ml).

Otherwise, the second assay was done according to [20] method described by [21] with difference in extraction buffer. Briefly, 0.5 ml of aluminium trichloride ($AlCl_3$) is added to 1 ml of extract. After 30 min of incubation at room temperature the sample absorbance was measured at 430 nm. The same procedure was repeated for the standard solution of rutin and calibration line was constituted. Based on the measured absorbance, the flavonoids concentration was read on the calibration line. The total falvonoids content was expressed as µg of rutin equivalent per mg of dry matter of the plant materials.

E. Antioxidant activity

The antioxidant activity of extracts was evaluated using DPPH scavenging assay. A 1 ml aliquot of extract was mixed with 950 µl of methanolic solution of DPPH in concentration of 1 mg/ml. The reaction mixture was shaken vigorously and incubated 30 min at room temperature. Then the absorbance at 517 nm was taken against a blank (DPPH solution without extract). The decrease in absorbance indicates the free radical scavenging effect of the tested sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged according to the following formula [22]:

$$P = \frac{(A1 - A2)}{A1} X100$$

P : Percentage of DPPH radical scavenged
A1 : control absorbance (DPPH solution without extract)
A2 : sample absorbance

F. Statistical analysis

Data were analyzed by one-way ANOVA test using STATISTICA software ver. 6 (Stat-Soft, 2001, France). Mean separations were performed by Newman and Keuls test, at $p < 0.05$.

I. RESULTS

A. Total phenolics content

The results obtained in this study showed a significant content of phenolic compounds in the majority of tested plants. Indeed, the amount of total phenolics in the 30 species varied from 2.54 to 55.62 µG GAE/ mg of dry matter (DM) (table 1). According to statistical analysis the investigated species were divided into several groups (table1). *Ceratonia siliqua extract (*55.62 µg GAE/mg) 1) exhibited the highest total phenolics content, followed by *Cistus villosus* (41.10µg GAE /mg), *Limoniastrum feei* (36.52 µg GAE /mg), *Rubus ulmifolius* (35.02µg GAE /mg), *Tetraclinis articulata* (34.71 µg GAE /mg) and *Rhus pentaphylla* (34.5 µg GAE /mg). Whereas, the lowest level of phenolics was found in *Conyza canadensis extract* (2.54µg GAE/mg).

Table 1: Percentage of antioxidant activity and total phenolics content (µg GAE/mg DM) of 30 analyzed species.

Botanical family	scientific name	Total phénolics	Antioxidant activity

Amaranthaceae	*Hammada scoparia*	25.88^{de}	88.21^{j}
Anacardiaceae	*Rhus pentaphylla*	34.50^{c}	92.17^{bcd}
Asteraceae	*Anvillea radiata*	11.54^{ijk}	88.57^{i}
	Artemisia herba-alba	19.86^{fg}	91.75^{cdef}
	Bubonium imbricatum	6.93^{lmn}	89.82^{hi}
	Conyza canadensis	2.54^{p}	88.19^{j}
	Inula viscosa	25.26^{de}	91.75^{cdef}
	Pulicaria dysenterica	7.81^{lm}	92.32^{bcd}
	Senecio anteuphorbium	3.99^{no}	91.26^{cdefg}
Brassicaceae	*Moricandia arvensis*	4.65^{mno}	90.50^{fghi}
Cistaceae	*Cistus villosus*	41.10^{b}	90.54^{fghi}
Cupressaceae	*Tetraclinis articulata*	34.71^{c}	94.17^{a}
Fabaceae	*Adenocarpus complicatus*	10.57^{jkl}	88.23^{j}
	Ceratonia siliqua	55.62^{a}	90.96^{defghi}
	Ononis natrix	13.86^{hij}	90.12^{ghi}
	Retama monosperma	12.55^{ij}	92.55^{c}
Lamiaceae	*Lavandula multifida*	16.38^{gh}	90.35^{fghi}
	Lavandula stoechas	23.71^{de}	93.49^{ab}
	Mentha pulegium	22.79^{def}	92.43^{cd}
	Teucrium chamaedrys	18.45^{g}	90.73^{efghi}
	Thymus leptobotrys	26.40^{d}	93.95^{a}
	Thymus pallidus	24.35^{de}	91.98^{cde}
	Thymus satureioides	18.81^{g}	92.24^{bcd}
Plantaginaceae	*Globularia alypum*	22.36^{ef}	88.34^{j}
Plumbaginaceae	*Limoniastrum feei*	36.52^{c}	90.01^{ghi}
Polygalaceae	*Polygala balansae*	14.69^{hi}	91.22^{cdefgh}
Rosaceae	*Rubus ulmifolius*	35.02^{c}	91.26^{cdefgh}
Rutaceae	*Ruta montana*	7.09^{lmn}	89.67^{i}
Solanacaea	*Witania adpressa*	8.64^{kl}	91.71^{cdef}
Verbenaceae	*Origanum vulgare*	15.05^{hi}	92.36^{bcd}

Numbers followed by a different letter are significantly different at P <0.05.

B. Total flavonoids content

Whatever the method being used, the content of flavonoids varied widely among tested species (table 2). Indeed, the method of [17] and [18] showed the highest amount of flavonoids in *Inula viscosa* (70.08 µg/mg DM) followed by *Globularia alypum* (66.28 µg/mg) and *Teucrium chamaedrys* (63.95 µg/mg). Other species had also high levels of flavonoids like *Ruta montana* (59.9 µg/mg), *Ononis natrix* (55.49 µg/mg), *Thymus satureioides* (53.79 µg/mg), *Polygala balansae* (49.66 µg/mg), *Artemisia herba-alba* (48.16 µg/mg), *Thymus leptobotrys* (40.63 µg/mg) and *Thymus pallidus* (40.24 µg/mg). While, in *Conyza canadensis* (5.41µg/mg) flavonoids were quit low.

The results obtained by [20] method are in agreement with those found with [17] and [18] assay. However, plants extracts with strong content of flavonoids using [20] method were *Rubus ulmifolius* (117.79 µg/mg DM), *Ononis natrix* (80.84 µg/mg DM), *Rhus tripartita* (67.67 µg/mg DM), *Thymus leptobotrys* (53.17 µg/mg DM) and *Thymus satureioides* (52.34 µg/mg DM). *Globularia alypum* (51.25 µg/mg DM), *Anvillea radiata* (48.20 µg/mg DM), *Ruta montana* (44.67 µg/mg DM), *Origanum vulgare* (43.17 µg/mg DM) and *Inula viscosa* (41.92 µg/mg DM) also had high levels of flavonoids. The lowest concentration was recorded in *Mentha pulegium* (9.46 µg/mg DM). Both methods gave the same results for 8 among 12 plants which had a high level of flavonoids content.

C. Antioxidant potential

The DPPH assay revealed that the extracts of 30 analyzed aromatic and medicinal plants have prominent antioxidant activity which varied from 88.19 to 94.17% (table 1). Indeed, a largest DPPH radical scavenging activity (up to 90%) was recorded in *Tetraclinis articulata* (94.17%), *Thymus leptobotrys* (93.95%) and *Lavandula stoechas* (93.49%). These results can be attributed to the higher phenolics content of the plants. Though, the antioxidant potential of Conyza canadensis (88.19%) was found to be relatively low. This species exhibited also the lowest content of total phenolics and flavonoids compared to the others investigated species.

Table 2: Total flavonoids content of 30 selected species (µg/mg DM)

Scientific name	Flavonoids (NEU)	Flavonoids ($AlCl_3$)
Hammada scoparia	9.9^{pq}	17.22^{no}
Rhus pentaphylla	24.39^{ik}	67.67^{c}
Anvillea radiata	30.28^{h}	48.20^{de}
Artemisia herba-alba	48.16^{f}	39.61^{fgh}
Bubonium imbricatum	11.71^{op}	23.14^{m}
Conyza canadensis	5.41^{q}	19.31^{mno}
Inula viscosa	70.08^{a}	41.92^{fg}
Pulicaria dysenterica	21.06^{jkl}	35.92^{hij}
Senecio anteuphorbium	14.24^{mnop}	14.67^{op}
Moricandia arvensis	12.28^{nop}	14.67^{o}
Cistus villosus	12.78^{nop}	33.59^{ik}
Tetraclinis articulata	17.84^{lmno}	37.14^{ghi}
Adenocarpus complicatus	38.8^{g}	34.42^{ij}
Ceratonia siliqua	20.41^{jklm}	23.42^{m}
Ononis natrix	55.49^{d}	80.84^{b}
Retama monosperma	20.37^{jklm}	15.92^{no}
Lavandula multifida	37.46^{g}	14.31^{o}
Lavandula stoechas	24.39^{g}	25.06^{m}
Mentha pulegium	24.39^{ijk}	9.46^{p}
Teucrium chamaedrys	63.95^{bc}	39.86^{fgh}
Thymus leptobotrys	40.63^{g}	53.17^{d}
Thymus pallidus	40.24^{g}	24.92^{lm}
Thymus satureioides	53.79^{de}	52.34^{d}
Globularia alypum	66.28^{ab}	51.25^{d}
Limoniastrum feei	6.46^{q}	13.56^{o}
Polygala balansae	49.66^{ef}	29.20^{kl}
Rubus ulmifolius	27.66^{hi}	117.79^{a}
Ruta montana	59.9^{c}	44.67^{ef}
Witania adpressa	18.15^{jlmn}	23.03^{m}
Origanum vulgare	19.4^{jklm}	43.17^{f}

Numbers followed by a different letter are significantly different at P <0.05.

II. DISCUSSION

The present study showed that the amount of total phenolics and flavonoids vary widely among the 30 investigated species. *Ceratonia siliqua* which had the highest content of phenolic compounds, was reported also to have a high level of catechic tannin and flavonoids [23, 24, 25]. Previous study [26], showed that *Limoniastrum feei*, in which the amount of phenolics was also high, contain gallic acid, quercetin and myricetin. Quantification of total flavonoids demonstrated that *Rubus ulmifolius* ([20] method) and *Inula viscosa* ([17] and [18]) method) were very high in flavonoids content. These two species were previously studied for their ethnobotanical use and their phenolics compounds content [27, 28, 29]. Thus [28] has reported that *Rubus ulmifolius* contains hydroxycinnamic acids (cafeic acid and ferulic acid), the caffeic acid esters, and flavonols (quercetin-3-O-glucuronid and kaempferol- 3-O-glucuronid). As *Rubus ulmifolius*, *Inulla viscosa* has also been reported to have the caffeic acid esters like chlorogenic acid [29] known for its prominent antioxidant potential.

For each plant, results of flavonoids quantification using two methods were different. This difference can be explained in the first hand by the dosage method used and the second hand by the nature of the used control. In fact, quercetin which is a flavonol aglycon, was used by [17] and [18] method to create a calibration line, while it was rutin (quercetin glycoside) in the method of [20]. However, statistical analysis demonstrated a positive correlation between these two methods. This finding is confirmed by quantification results obtained in 8 plants and for which the concentrations of flavonoids were high with both methods. These plants are: *Ononis natrix*, *Thymus leptobotrys*, *Thymus satureioides*, *Globularia alypum*, *Ruta montana*, *Artemisia herba-alba*, *Anvillea radiata*, *Teucrium chamaedrys* et *Inula viscosa*. Among 15 families tested in this study, Lamiaceae and Asteraceae are the most representative. These two families are known to be rich in secondary metabolites and to be widely used in natural phytotherapy [30].

The high phenolics content of investigated plants play a considerable role in their antioxidant action. Indeed, phenolics compounds are a major class of antioxidant agents because of their scavenging ability on free radicals [31]. Due to their several hydroxyl groups, flavonoids have been shown to be highly effective scavengers of various free radicals implicated in several diseases [32, 33].

According to [34], the method using DPPH, a free and stable radical, is a simple and rapid way to study the antioxidant capacity of a plant extract. Results obtained in our study showed that the majority of tested plants had a prominent antioxidant activity, which is up to 90%. Several researchers have previously reported the high linear correlation between the total phenolics content and the antioxidant activity [35, 36, 37, 38, 39, 40]. Generally, plants with great amount of phenolics compounds had a very strong antioxidant capacity.

This radical scavenging activity can be affected by to substitution of hydroxyl groups on phenolics aromatic ring, because of their hydrogen donor ability [41, 13]. In fact, the antioxidant activity increase with increasing degree of hydroxylation, however substitution of the hydroxyl groups with methyl (CH_3), methyoxyl (OCH_3) groups…etc, reduces the activity. So, his can explain the important antioxidant potential observed in some tested aromatic and medicinal plants compared to others.

III.CONCLUSION

The main objective of our study was to determine the total phenolics compounds as well as to evaluate the antioxidant activity of 30 plants from Southern Morocco flora, which are used in traditional medicines.

Results of phenolics and flavonoids quantification demonstrated that the majority of analyzed species are rich in phenolics compounds which have been shown to exert scavenging free radicals activity.

In addition, this study noticed the linear correlation between the values of phenolics compounds and antioxidant potential which indicates the contribution of these compounds to the strong antioxidant activity. Indeed, more than 2/3 of analyzed plants had up to 90% of antioxidant potential.

The phytochemical investigation of the 30 plants, collected from 3 localities which are different by their climate, altitude…etc, and some of which are endemic such *Bubonium imbricatum* , *Senecio anteuphorbium* , *Thymus leptobotrys*, may add value to existing data on the role of phenolic compounds in the oxidative stress regulation.

ACKNOWLEDGEMENTS

This paper is homage to all Moroccan rural population. The authors thank the rural population of the 3 localities (Agdz, Tafraout and Taroudannt) for their help and their warm hospitality.

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