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Anti-glycation and radical scavenging activities of hydro-alcohol and aqueous extracts of nine species from *Lamiaceae* family

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Abstract
Antioxidant and anti-glycation properties of nine Moroccan plants of the *Lamiaceae* family used in traditional medicine, has been evaluated by after extraction by hot (HAE) or cold (CAE) maceration and by ethanol (EE). Anti-glycation activity was performed on BSA-Methylglyoxal system and was measured by fluorescence and native electrophoresis.
The EE yielded the highest levels of polyphenols and flavonoids at *Origanum Compactum*. The same extract showed a strong antioxidant activity with IC_{50} = 75.5±0.63 µg/ml, followed by *Rosmarinus officinalis* with IC_{50} = 99.7±0.47 µg/ml, and *Calamintha officinalis* with IC_{50} = 111±0.5 µg/ml. All plants studied showed dose dependent anti-glycation activity, with a pronounced effect recorded in *Thymus satureioides*. The aqeous extracts showed a correlation between polyphenols and flavonoids witch was in turn correlated with antioxidant activity. No correlation was observed between anti-glycation and anti-oxidation/polyphenol content. The *Lamiaceae* family is therefore a rich promoter source in phenolic compounds, and presents both an antioxidant and anti-glycation activity.

Keywords: Anti-glycation, antioxidant, polyphenols, flavonoids, water extracts, ethanol extracts.

Introduction
For centuries, humans used plants to relieve his pain, heal its ills and wounds. This knowledge have been passed from generation to generation, and this traditional medicine has been documented [1]. With technological and scientific progress, drugs based on chemical molecules substituted quickly the use of plants [2].
Diabetes is a very common metabolic disease in the world that affects over 365 million people [3]. Actually, this number tends to increase towards the year 2030. In Morocco, the number of diabetics exceed 2.5 million, which mean 7.8% of the population [4]. Diabetes is indeed a handicap for patients once associated with complications [5, 6]; the most common are microvascular, macrovascular and cerebrovascular damage [7]. Their triggering is explained by four hypotheses: The increase of polyol pathway, activation protein kinase, oxidative stress and protein glycation.
Protein glycationone of the main causes of these complications, is a non-enzymatic reaction that takes place between the reducing sugars and the free amino functions of proteins, resulting in the accumulation of toxic products - known as advanced glycation end products (AGEs) - in the tissues due to a reaction called "Maillard reaction" or glycation. AGEs alter the physicochemical properties of proteins, making them non-functional. In addition, the binding of AGEs in their RAGEs receptors is involved in the development of diabetic complications. Coupled to oxidative stress and free radicals generated, the situation becomes critical and imposes a need to discover new compounds with anti-glycation and anti-oxidation effects [8].
There are a variety of compounds used to reduce glycation and its deleterious effects. These products include metformin pyridoxamine, benfotiamine, the OPB-9195 [9], etc. However, these treatments have several disadvantages such as the effects secondary. To seek natural molecules in order to solve the problem of glycation, a great interest has been allocated for medicinal and aromatic plants from Morocco. This country has a real phytogenetic tank (with about 4,500 species and sub species of vascular plants), and an important place among the Mediterranean countries with long tradition and expertise in medical herbal [10].
In this study, ethanol and aqueous extraction of nine medicinal and aromatic plants from the *Lamiaceae* family were compared for polyphenols and flavonoids content. The *in vitro*

evaluation of their anti-oxidation and anti-glycation effects were then performed. It was shown that the polyphenol content depends on the plant used and the type of extraction. The polyphenol content was significantly correlated with the antioxidant effect, while there is no correlation between these two parameters and the anti-glycation effect.

Material And Methods

1. Chemicals

All reagents (Ethanol, Methanol, Sodium carbonate (Na_2CO_3), Sodium bicarbonate, Folin–Ciocalteau reagent, Gallic acid, Aluminum trichloride, Potassium acetate, Quercetin, Iron II ($FeCl_2$), Ferrozine, BSA, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methylglyoxal 40%, copper sulfate, sulfuric acid, anhydrous sodium sulfate, potassium sodium tartrate, arsenomolybdate and glucose) unless otherwise stated, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Metformin is commercialized as Glucophage 500 mg (purity 78%).

2. Plant collection and extract preparation

Plants are from different regions of Morocco, and were collected in March 2015 (Table 1). The selected parts of the plants were dried at 40 °C in an oven for 15 h. All samples were then ground into a fine powder. The powders were passed through an 80-mesh sieve, collected and then stored at 20 °C until use. Aqueous extracts were obtained by extraction of samples (30 g) with distilled water (300 ml) for 45 min, three times, at 80 °C (HAE) or at 25 °C (CAE). Hydro-alcohol extract was obtained by extraction of samples (20 g) with 200 ml of ethanol solution (70%) for 24h, three times. The macerates were filtered and centrifuged for 20 min (4000 t/min) at room temperature. After evaporation of supernatants, the extracts obtained were stored at 4°C away from light until use.

Table 1: Plants description

Botanical name	Common name	Genre	Origin	part used	Harvest period	wild / cultivated plant
Calamintha officinalis	Manta	*Calamintha*	Marrakech	Leaves	May	Wild
Lavandula dentata	Khzama Beldia	*Lavandula*	Marrakech		March	Wild
Mentha pulegium	Flio	*Mentha*	Marrakech		March	Wild
Mentha routundifolia	Marceta	*Mentha*	Marrakech		March	Wild
Origanum compactum. Benth	Zaâtar	*Origanum*	Marrakech		March	Wild
Origanum majorana	Merdeddouch	*Origanum*	Tangier		March	Cultivated
*Rosmarinus officinalis.*L	Azir	*Rosmarinus*	Marrakech		Jun	Wild
*Salvia officinalis.*L	Salmia	*Salvia*	Marrakech		March	Cultivated
Thymus satureioides	Zâitra	*Thymus*	Marrakech		March	Wild

The following formula was used to determine the extracts yield (11): $R = (P_x / P_y) * 100$

R : Extract yield (%), P_x : Extract weight (g), P_y : Plant weight (g).

3. Quantification of polyphenols, flavonoids and reducing sugars

3.1 Determination of Total Phenolic Contents (TPC)

Amount of TPC was determined by Folin-Ciocalteu method (12). Briefly, 100 µl of extracts (1 mg/ml) were added to 500 µl of 1:10 Folin–Ciocalteau reagent (prepared prior to use). After 4 min, 400 µl of sodium carbonate 7.5% (m/v : 75mg/ml) were added. After 30 min of incubation at room temperature, the optical density at 765 nm was measured by spectrophotometer type VARIAN Cary 50 UV/Vis. The standard range was prepared from a solution of Gallic acid (GA) (5 mg/ml) with concentrations ranging from 0 to 150 µg/ml. The results were reported in Gallic Acids Equivalents (GAE) per g of sample.

3.2 Determination of Total Flavonoid Contents (TFC)

The TFC was determined byaluminum trichloride colorimetric method (AlCl3) (13), with modifications. Briefly, 250 µl of extracts (2 mg/ml) were added to 1.4 ml of deionized water, 50 µl of potassium acetate (1 M), 50 µl of aluminum trichloride 10% (m/v), and 750 µl of absolute ethanol. After 30 min of incubation at room temperature, the absorbance at 415 nm was measured (VARIAN Cary 50 UV-Vis).The standard range was prepared from a solution of Quercetin (10 mg/ml of ethanol 80%), with concentrations ranging from 0 to 150 µg/ml. The results were reported in Quercetin Equivalents (QE) per g of sample.

3.3 Determination of Reducing Sugar Content

The reducing sugar content was determined by Nelson-Somogyi method [14], with modifications. Briefly, 100 µl of extracts (1 mg/ml) were added to 1.9 ml of ultrapure water, and 1 ml of alkaline copper tartrate reagent. After 10 min of incubation at 100 °C, the arsenomolybdic reagent was added. The volume was completed to 10 ml with distilled water and the absorbance at 620 nm was measured. The standard range was prepared from a glucose solution (100 mg/ml), with concentrations ranging from 0 to 100 mg/ml. The results were reported in Glucose Equivalents (GE) per g of sample.

4 Antioxydant activity

4.1 Radical DPPH-scavenging activity

Free radical-scavenging capacities of extracts were determined according to Braca, 2002 [15], with some modifications. For this, 4 mg of DPPH were dissolved in 100 ml of methanol and incubated in the dark for 3H before use. Briefly, 250 µl of extract (0.06, 0.125, 0.25, 0.5, 1 and 10 mg/ml) were added to 750 µl of DPPH solution and then incubated for 30 min in the dark. The absorbance was measured at 517 nm against a control of methanol and DPPH solution (250 µl for 750 µl respectively). The standard range was prepared from a solution of ascorbic acid (5 mg/ml), with concentrations ranging from 0 to 500 µg/ml. The anti-radical activity was estimated by the following equation :

% of anti-radical activity = [(Abs_{517} control –Abs sample$_{517}$) / Abs_{517} control] x 100

4.2 Ferric-reducing antioxidant power assay (FRAP assay)

The chelating capacity of extracts was measured according to Chiu *et al.,* 2007 [16] with modifications. Briefly, 250 µl of extracts (0.25, 0.5, 1, and 10 mg/ml) was added to 50 µl of FeCl2 (0.6 mM) and 450 µl of methanol. After 5 min, 50 µl of Ferrozine (5 mM) was added. After 10 min of incubation at room temperature, the absorbance at 562 nm was measured (VARIAN Cary 50 UV-Vis). Methanol replaced the extract in

negative control. The standard range was prepared from a solution of Quercetin (10 mg/ml of ethanol at 80%), with concentrations ranging from 0 to 150 µg/ml. Percentage of chelation was calculated by the following equation:
% Chélation = [(Abs562 contrôle – Abs562 échantillon) / Abs562 contrôle] x 100

5 Antiglycation activity

5.1 *In vitro* glycation of serum bovine albumin

Bovine serume albumun (BSA 5 mg/ml, containing EDTA) was incubated with methylglyoxal (10 mM) and sodium azide (0.02%) in 0.1 M phosphate buffer (pH 7.4). Three concentrations of tested compounds (1.5, 3.5 et 10 mg/ml) were added to the reaction mixture, then incubated for 24 h at 50°C away from light and stirred. Individual vials were removed at desired times and stored frozen at 20°C until analyzed. Metformine (30 mM) was used as positive control.

5.2 Electrophoretic migration in native conditions

The mixture solution was applied to PAGE-Native. The samples were separated on a 7% polyacrylamide gel. After migration, the gels were stained with coomassie blue for 1 h. the destaining was also for 1 h with a solution of acetic acid 10% and methanol 45%.

5.3 Spectrofluorimetric measure

Fluorescence measure was performed in the chemistry laboratory of the National Office of food safety of Tangier (ONSSA). Comparison the fluorescence spectrum (excitation at 370 nm) and the change in fluorescence intensity (excitation at 370 nm and emission 423 nm) was performed through a spectrofluorimeter type (VARIAN Cary 50 UV-Vis).

6 Statistical analysis

The results of *in vitro* tests were expressed as mean ± SD. The difference between the control and samples was determined by uni-varied ANOVA followed by Fischer's test. The p values ≤0.05 were considered significant.

Results And Discussion

1. Yields and pH of extracts

The extraction of phenolic compounds allowed us to calculate the yields for the different extracts. Below is a summary table of yields and pH of our extracts (Table 2).

Table 2: Yields and pH of extracts

Plants	Yields %			pH		
	HAE	CAE	EE	HAE	CAE	EE
Calamintha officinalis	6.5	8.5	15	5.47±0.23	5.63±0.68	6.16±0.78
Lavandula dentata	7.9	5.3	4	7.26±0.56	6.46±0.28	5.59±0.44
Mentha pulegium	8	5.2	5.8	6.71±0.34	6.84±0.56	6.23±0.09
Mentha rotundifolia	8.225	8	8	5.37±0.05	5.92±0.07	5.37±0.78
Origanum compactum. Benth	7.95	9	17.4	7.66±0.11	7.48±0.80	5.87±0.1
Origanum majorana	6.27	7.73	7.02	6.73±0.32	6.27±0.94	6.25±0.71
Rosmarinus officinalis. L	8.55	8.35	9.6	6.27±0.97	6.59±0.03	5.37±0.32
*Salvia officinalis.*L	8.95	8.35	22	6.21±0.12	6.64±0.12	6.08±0.76
Thymus satureioides	6.5	5.65	10.8	6.05±0.34	5.53±0.34	6.67±0.38

The results showed that the pH of the extracts varied between 5.37 and 7.66 (n = 3). Numerous studies have correlated the pH value of an aqueous extract to reactions kinetic laws, organoleptic qualities, or enzymatic activities [17], this pH is measured to allow the interpretation of certain biological activity results [18].

It was also found that ethanol extracts have higher yields compared to hot and cold aqueous extracts. A maximum value was observed in *S. officinalis* (22%) followed by *O. compactum* (17.4%) and *C. officinalis* (15%). The minimum values were represented by *M. pulegium* (5.8%) followed by *L. dentata* (4%). Regarding the cold aqueous extracts, they have the lowest yields with 9% as maximum value observed in *O. compactum*, and 5.2% as minimum value for *M. pulegium*. Yields in hot aqueous extracts were slightly larger than cold aqueous extracts; the maximum value was represented by *S. officinalis* with 8.95%, while the minimum was observed in *O. majorana* with 6.27%.

Comparing our results with those of the literature seems difficult, because the yields varies depending on the solvent [19], the plant organs [20] and from one species or family to another [21]. Indeed, it is correlated with the genetic characteristics of MAPs, their geographic origin, the harvest period, the conditions and duration of storage and finally the method and duration of maceration [4].

The extraction presents a crucial step before any manipulation or analysis of MAPs. In this study, the ethanol extraction was observed ideal in terms of dry matter yield, followed by hot aqueous extraction. According to Mannan (2014) [22], for 141 anti-diabetics plants, 39.7% of extractions are performed by aqueous solvent while only 25.5% are performed by ethanol. In fact, ethanol is more privileged compared to other solvents because of its acceptance for animal and human consumption [20].

2. Total Phenolic, Flavonoid and Reducing sugar contents

2.1 Total Phenolic Contents

The determination of total polyphenols was performed by the Folin-Ciocalteu method with gallic acid as standard. The regression equation of the standard curve was: Abs = 0.00448*concentration, with $R^2 = 0.9811$.

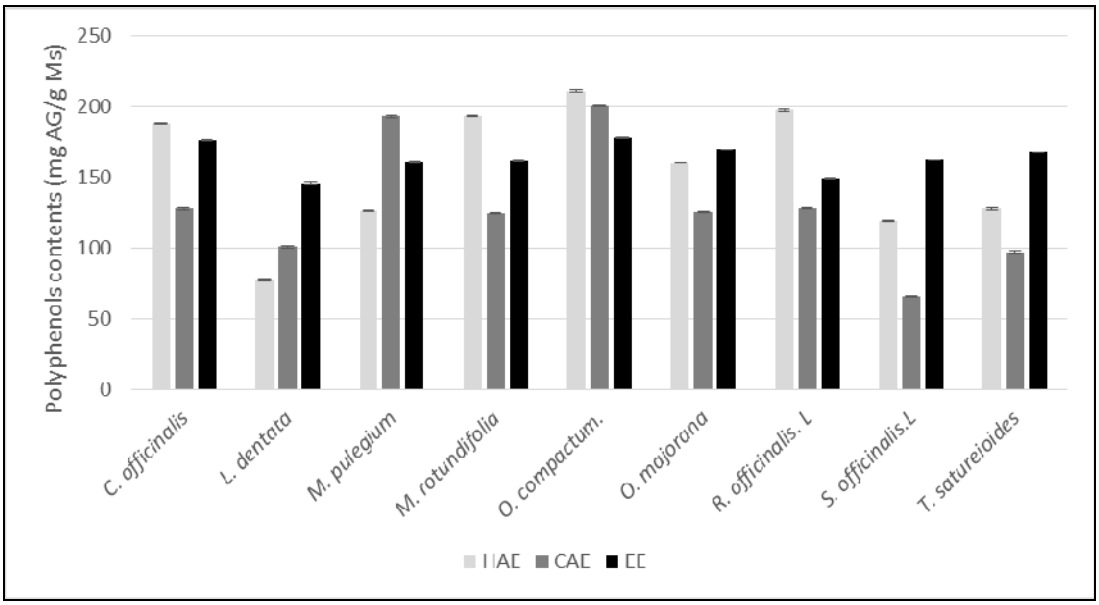

Fig 1: Polyphenols Contents in mg GAE/g Ms

The HAE and EE were extracts that shown the highest polyphenols values (Fig. 1). The highest polyphenol content was recorded in *O. compactum* with 178.35 ± 0.13 mg GAE/g Ms for EE and 211.60 ± 0.19 mg GAE/g Ms for HAE, followed by *R. officinalis* with 176.68 ± 0.45 and 197.36 ± 0.25 mg GAE/g Ms for EE and HAE respectively. The lowest polyphenol content was recorded in *L. dentata* with 145.61 ± 0.23 mg GAE/g Ms for EE, and only 77.49 ± 0.15 mg GAE/g Ms for HAE. Concerning CAE, the highest levels were recorded in *O. compactum* by 200.52 ± 0.56 mg GAE/g Ms followed by *M. pulegium* with 193.57 ± 0.77 mg GAE/g Ms. While the lower content was that of *S. officinalis.* L with 65.61 ± 0.36 mg GAE/g Ms.

2.2 Total Flavonoid Contents

The flavonoids dosage was performed according to aluminum trichloride method, with quercetin as standard. The regression equation of the standard curve was Abs = 0.00253 * concentration, with $R^2 = 0.9984$.

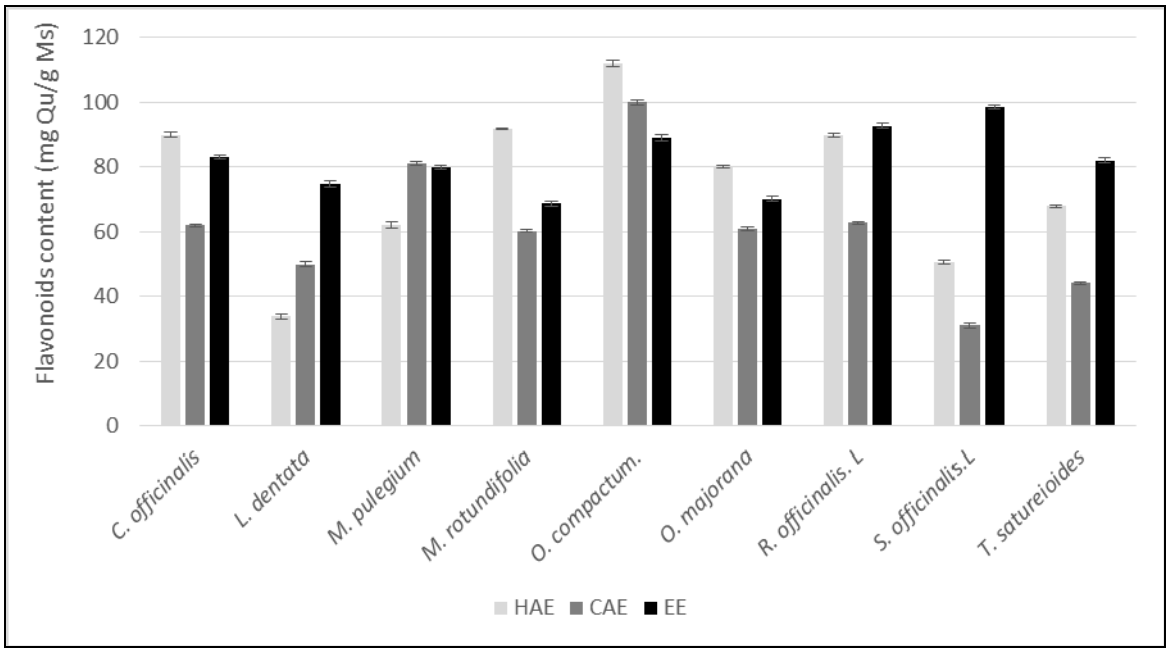

Fig 2: Flavonoids contents in mg Qu/g Ms

Flavonoids are the largest polyphenolic class, with more than 5,000 compounds already described [23]. In general, it was found that the flavonoid contents varied from a sample to another (Fig. 2), but still lower than those of total polyphenols (Table 2). For EE, these contents were between 68.22 ± 0.17 mg GAE/g Ms in *M. rotundifolia* and 98.18 ± 0.20 mg GAE/g Ms in *S. officinalis*. For HAE, they ranged from 33.86 ± 0.25 mg Qu/g Ms in *L. dentata* and 112.52 ± 0.20 mg Qu/g Ms in *O. compactum*. Concerning CAE, the most remarkable value was recorded in the case of *O. compactum* with 100.61 ± 0.21 mg Qu/g Ms. While *S. officinalis* recorded only 30.45 ± 0.18 mg Qu/g Ms.

From the results of table 3, the difference of phenolic and flavonoid content between those plants was small in the three extract type, up to 3 fold. The differences between these results and the literature can be attributed to intrinsic (plant genetics) and extrinsic factors (environmental, extraction protocol, preservation and storage...) [24, 25].

Table 3: Comparative study of polyphenol and flavonoid concentrations of hot, cold and ethanolic extracts, between the nine species of the Lamiaceae family.

Plants	Polyphenols (mg AG/g Ms)			Flavonoids (mg Qu/g Ms)			Correspondance in literature		
	MEAN HAE ± Er.Std	MEAN CAE ± Er.Std	MEAN EE ± Er.Std	MEAN HAE ± Er.Std	MEAN CAE ± Er.Std	MEAN EE ± Er.Std	Polyphenols (mg AG/g Ms)	Flavonoids (mg Qu/g Ms)	Reference
C. officinalis	188,71±0,18 (f)	127,34±0,23 (c)	176,44±0,20 (g)	90,682±0,31 (g)	62,255±0,18 (d)	83,449±0,24 (e)	-	-	-
L. dentata	77,49±0,15 (a)	100,84±0,22 (b)	145,61±0,23 (a)	33,866±0,25 (a)	50,668±0,24 (c)	74,447±0,44 (c)	-	-	-
M. pulegium	126,28±0,28 (c)	193,48±0,37 (d)	160,47±0,20 (c)	62,48±0,26 (c)	81,669±0,28 (e)	80,349±0,15 (d)	0.338 6.1 ± 0.5 206.58± 4.54	0.037 0.85 ± 0.01 77.12± 2.93	(Ghazghazi. 2013) (Fatiha et *al*.. 2015) (Yumrutas. 2012)
M. routundifolia	193,46±0,24 (g)	124,31±0,20 (c)	162,48±0,10 (d)	92,677±0,20 (h)	60,311±0,19 (d)	68,223±0,17 (a)	4.6 ± 0.1 (EE)	3.3 ± 0.1 (EE)	(Fatiha et *al*.. 2015)
O. compactum	211,60±0,19 (i)	209,60±8,93 (e)	178,35±0,13 (h)	112,527±0,20 (i)	100,613±0,21 (f)	89,338±0,22 (f)	-	-	-
O. majorana	160,36±0,16 (e)	125,41±0,15 (c)	170,32±0,18 (f)	79,557±0,18 (e)	61,73±0,22 (d)	70,571±0,40 (b)	4.65±1.00 (EE)	-	(Roby et *al*.. 2013)
R. officinalis	197,36±0,25 (h)	127,76±0,26 (c)	148,47±0,21 (b)	89,564±0,22 (f)	62,451±0,14 (d)	92,407±0,18 (g)	24.94±0.17 (HAE) 190 (EE)	18.83±0.3 (HAE) -	(Chen et *al*.. 2015) (Kozłowska et *al*.. 2015)
S. officinalis	119,11±0,20 (b)	65,29±0,22 (a)	163,37±0,73 (d)	50,558±0,19 (b)	30,459±0,18 (a)	98,189±0,20 (h)	185 (HAE) 5.8±1 (EE)	- -	(Dorman et *al*.. 2003) (Roby et *al*.. 2013)
T. satureioides	127,48±0,09 (d)	97,22±0,14 (b)	167,32±0,14 (e)	67,255±0,20 (d)	40,361±4,04 (b)	82,672±0,29 (e)	475.00 ± 8.30 456.73±6.94	182.79 ± 3.23 172.79±2.12	(Khouya et *al.*, 2015) (Ramchoun et *al.*, 2015)
TOTAL	155,76±4,54	130,14±4,64	163,65±75,46	75,4628±61,16	61,1685±2,12	82,1827±1,01	----		
FISHER	50687,39 (p<0,000) **	20679,23 (p<0,000) **	1256,25 (p<0,000) **	232,01 (p<0,000) **	4332,87 (p<0,000) **	1008,35 (p<0,000) **			

Groups with the same letters do not differ significantly by tukey test; Er.Std: Standard Error; HAE: Hot Aqueous Extract; CAE: Cold Aqueous Extract; EE: Ethanolic extract; **: very highly significant difference**.**

According to Chanda et *al.,* (2009) [26], solvent polarity plays an important role in in the phenolic compounds solubility, while warm maceration can lead to the depletion of phenolic compounds present in the extracts [27, 28]. The results of Mohsen & Ammar, (2009) [29] showed that ethanol was the best solvent for the extraction of phenolic compounds, followed by methanol and finally water. However, this has not been elucidated in our experiments, where HAE has shown significant polyphenol and flavonoid levels as important as those obtained by EE (Table 2). This can be explained by two factors: firstly, the short extraction time (1 h à 80 °C) does not permit the regression of phenolic compounds levels; secondly, the volume of solvent, which has been used in the hot-maceration (200 ml), allows better penetration into the vegetable matrix and a strong release of the phenolic compounds. In addition, levels of polyphenols and flavonoids recorded by EE remained slightly larger compared to the CAE. This can be attributed to the poor performance of this cold maceration technique because of the low temperature that was not suitable for the release of bioactive compounds. Then, duration of the extraction must be extended to achieve good results. In addition, its beneficial health effects are not well studied [30].

From these data, within the same family, each plant has an extraction method for releasing the maximum of phenolic compounds. Therefore, it would be difficult to standardize a valid extraction protocol for all MAPs [31]. The quantification of polyphenols by Folin-Ciocalteu method has not illustrated the real picture of quality and quantity of phenolic constituents of our extracts, because of the complexity and difference of an extract to another [32]. It is therefore necessary to couple this assay to the HPLC technique [33].

The study of the correlation between the polyphenols and flavonoids contents of the three types of extracts showed that the HAE (R^2 = 0.952) and CAE (R^2 = 0.9542) were proportional and significantly correlated (p<0.05) (Figure 5), which informs that flavonoids are the major fraction in these two types of extracts.

Maisuthisakul et *al.,* (2008) [34] have found that the total flavonoids content of EE of 28 plants was positively correlatedto the total phenolic compounds content. Thus, for EE, the literature results does not corroborate our study that shows no correlation (R^2 = 0.0004), suggesting that the fraction of flavonoids represented a minority. The choice of these substances is based on the fact that the majority of anti-oxidant properties of plants is attributed to allocated to them.

2.3 Reducing sugar content

The reducing sugar assay was performed according to Nelson-Somogyi method, with glucose as standard. The regression equation of the standard curve was y = 0.0976 x + 0.0506 with R^2 = 1

Table 4: Comparative study of reducing sugars concentrations of hot, cold and ethanolic extracts, between the nine species of the Lamiaceae family**.**

Plants	Reducing sugars (mg EG/g Ms)		
	MEAN HAE ± Er.Std	MEAN CAE ± Er.Std	MEAN EE ± Er.Std
C. officinalis	16,126±0,29 (a)	14,911±0,35 (b) (c) (d)	13,031±0,48 (b) (c)
L. dentata	16,802±0,24 (a) (b)	16,039±0,43 (c) (d)	12,088±0,34 (a) (b)
M. pulegium	18,399±0,23 (c)	16,45±0,49 (d)	13,078±0,44 (b) (c)
M. routundifolia	16,756±0,33 (a) (b)	14,648±0,073 (b) (c)	12,753±0,33 (a) (b)
O. compactum	15,735±0,252 (a)	12,255±0,67 (a)	11,459±0,18 (a)
O. majorana	16,916±0,25 (a) (b)	14,669±0,41 (b) (c) (d)	12,57±0,32 (a) (b)
R. officinalis	17,368±0,21 (b) (c)	13,361±0,32 (a) (b)	14,322±0,26 (c)
S. officinalis	16,684±0,25 (a) (b)	14,992±0,26 (b) (c) (d)	12,609±0,21 (a) (b)
T. satureioides	15,981±0,27 (a)	14,689±0,23 (b) (c) (d)	12,158±0,24 (a) (b)
TOTAL	16,751±0,11	14,668±0,17	12,674±0,13
FISHER	9,34 (p<0,000) **	10,05 (p<0,000) **	5,84 (p<0,000) **

Groups with the same letters do not differ significantly by tukey test; Er.Std: Standard Error; HAE: Hot Aqueous Extract; CAE: Cold Aqueous Extract; EE: Ethanolic extract; **: very highly significant difference.

From the results of Table 4, it was found that the reducing sugar content varied from one plant to another and depending on the type of extract. In general, the HAE presented the highest contents followed by CAE and EE, with minimum and maximum values recorded respectively in *O. compactum* and *M. pulegium*. These results are in agreement with other studies showing that carbohydrates are present among other phytochemical constituents of these extracts [35, 36].

During hot aqueous extraction, the action of heat causes the hydrolysis of sucrose which produces reducing sugars; this hydrolysis is less in absence of heat in cold aqueous extraction. These intrinsic carbohydrates may indirectly induce fluorescence [37]. According to Arhab R., (2007) [38], the total sugar content varies depending on the climate, season and stage of plant development.

1. Antiradical activity

1. 1The free radical DPPH scavenging

To highlight extracts with radical scavenging power, the maximum inhibitions percentages and the IC_{50} were determined. Ascorbic acid has recorded a powerful anti-radical activity with an IC_{50} of 4.8±0.0005 mg/ml and a maximum inhibition percentage of 98.72%, higher than the three extracts types of the nine plants studied in this work (Figure 3, Table 3).

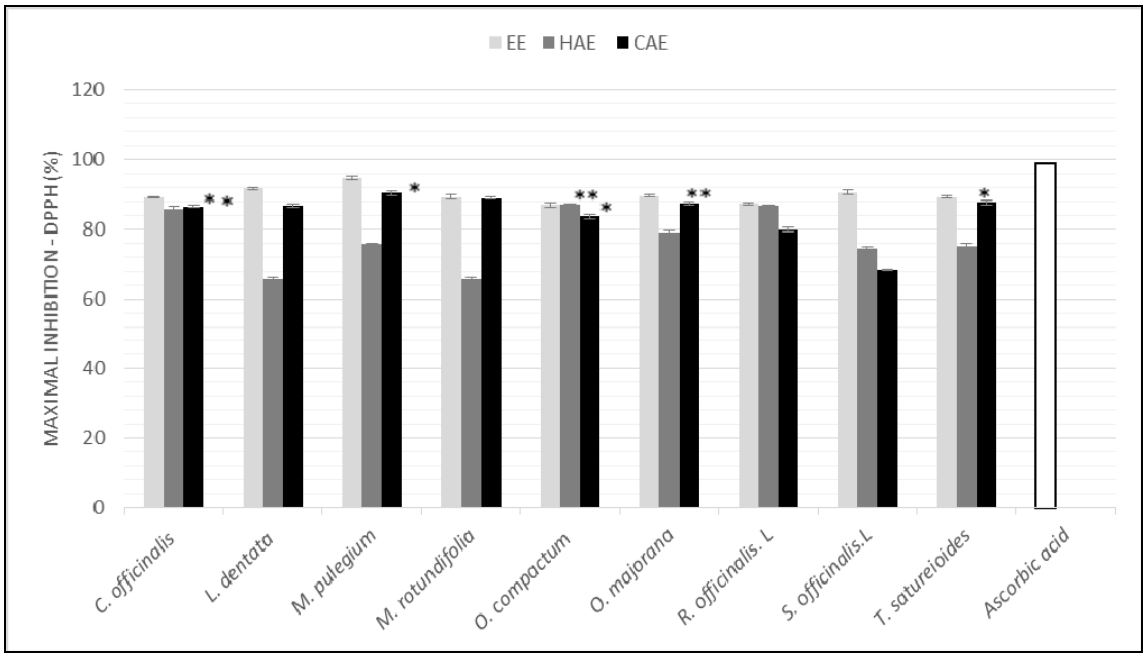

Fig 3: Maximal percentages of DPPH radical scavenging of EE, HAE and CAE. The results are expressed as mean ± SD (n = 3); ** Indicates a highly significant difference 0.0005 <*P*<0.005; * Indicates significant difference 0.005 <*P*<0.05

From these data, it was found that, for these plants belonging to the *Lamiaceae* family, the EE followed by CAE had an anti-radical activity greater than 80%, which is closer to the positive control (Ascorbic acid). This activity could be related to their high polyphenol content. There was significant differences ($p<0.05$) between the IC_{50} of the EE and HAE, and between the highest inhibition percentages of the 3 types of extracts and the 25 plants.

For EE, the IC_{50} ranged from 92.2 to 762 µg/ml. Plants representing significantly low IC_{50} values were *C. officinalis* and *O. majorana*, with 201±0.64, 620±0.205 and 215±0.36 µg/ml respectively. The percentage inhibition of the DPPH radical was between 87.05 and 94.84%. The three maximum values were recorded in the case of *M. pulegium* followed by *L. dentata* and *S. officinalis* with 94.84, 91.75 and 90.52% respectively.

The HAE showed IC_{50} values ranging from 75.5±0.63 µg/ml in *O. compactum* to 345±0.34 µg/ml in *T. satureioides*. The extract of *M. rotundifolia* presented also a significantly lower IC_{50} with 104±0.63 µg/ml. While the percentage of maximal inhibition varied from 65.77% in *M. rotundifolia* to 87.18% in *O. compactum*.

Concerning CAE, IC_{50} values were ranging from 71.2±0.25 µg/ml in *O. compactum* and 92.9±0.1 µg/ml in *M. pulegium,* to 751±0.63 µg/ml registered in the case of *S. officinalis*. Nevertheless, the most significant value was that of *R. officinalis* with 335±0.36 µg/ml. in addition, *M. pulegium* followed by *T. satureioides* were recorded the highest percentages of inhibition with 90.34% and 87.68% respectively. While *S. officinalis* had only 68.31% inhibition.

Generally, it seems that the three types of extracts had a good scavenging power of DPPH radical, but EE stilled the best. These results were consistent with those of Sultana et *al.,* (2009) [28]. However, ascorbic acid and extracts were both unable to reduce the totality of DPPH radical. Moreover, there was a dose-response effect for the three types of extracts, which explains the activity increase by raising levels of phenolic compounds.

According to Turkmen et al., (2007) [39], polyphenols seem to be an effective donor of hydrogen to the DPPH radical, due to their ideal structural chemistry. Nevertheless, the results of this study appear contradictory. On one hand, HAE showed the highest levels of polyphenols and flavonoids. On the other, the EE has the best scavenger power of free radicals. This can be explained partly by the fact that not all phenolic compound includes in the HAE contributes to trapping DPPH radical, and that each phenolic compound has a specific antioxidant activity [40]; secondly, by the synergy between the mixture of phenolic compounds present in the same extract which not only depend on their concentration but also their structure [41, 42]. Other phenolic compounds should not be neglected, because the synergy between different chemicals should be considered in the biological activity [43].

1.2 The ferrous iron chelation

To highlight the extracts able to break the ferrous iron-ferrozine complex, percentages of maximum chelation and IC_{50} were determined. Quercetin showed strong chelating activity with an IC_{50} of 5.4±0.0012 µg/ml and 93.15% as maximum percentage of chelation. Thus, these values remain higher than those of all plants studied (Figure 4).

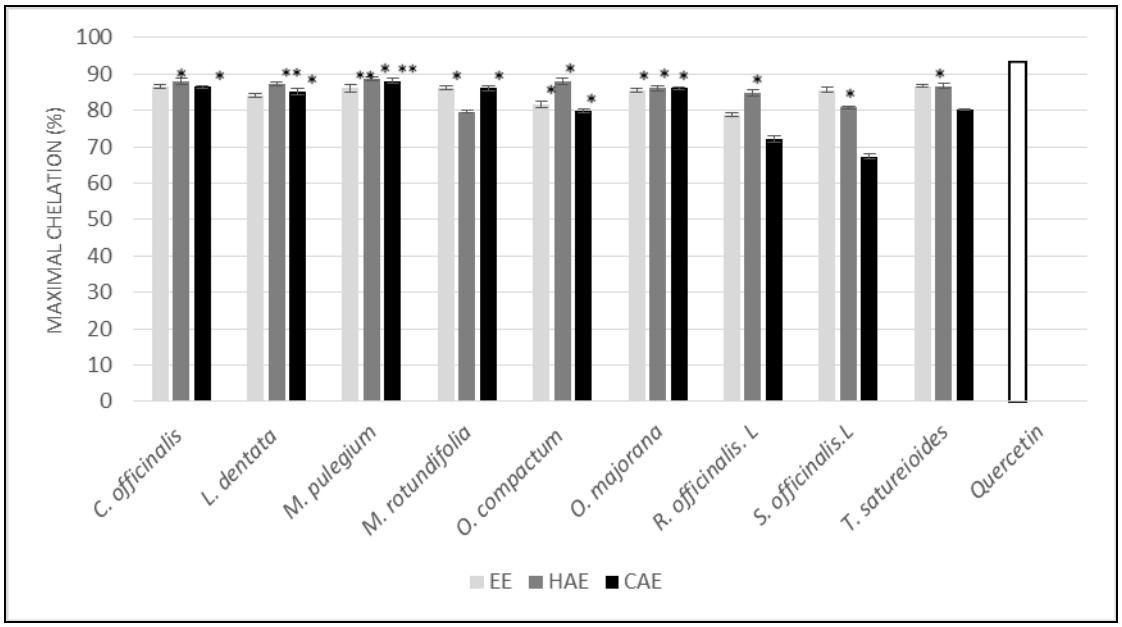

Fig 4: Maximal chelation percentages of EE, HAE and CAE. The results are expressed as mean ± SD (n = 3); ** Indicates a highly significant difference 0.0005 <*P*<0.005; * Indicates significant difference 0.005 <*P*<0.05

IC_{50} values of EE were between 281±0.63 µg/ml in *C. officinalis* and 348±0.31 µg/ml in *T. satureioides*. While the maximal percentages of chelation were registered in *T. satureioides, C. officinalis, M. routundifolia* and *M. pulegium*, with 86.64, 86.54, 86.17 and 86.13% respectively. The lower percentage was presented in *R. officinalis* with 78.82%.

For HAE, the IC_{50} were ranging from 245±0.42 µg/ml in *C. officinalis,* followed by *R. officinalis* with 274±0.46 µg/ml to 972±0.68 µg/ml in *L. dentata*. The maximal percentages of chelation were those of *M. pulegium,* followed by *O. compactum* and *C. officinalis* with 88.7, 88.06 and 88.03% respectively. While *M. rtundifolia* presented only 79.67%.

Concerning CAE, the most interesting IC_{50} values were those of *O. compactum, M. routundifolia* and *M. pulegium*, with 276, 356 and 357 µg/ml respectively. *M. pulegium, C. officinalis* and *M. rotundifolia* presented interesting percentages of chelation, with 88.11, 86.54 and 86.09%, and a minimal value in the case of *S. officinalis. L* with 67.35%. In general, the difference of antioxidant capacities between those plants was small in the three extracts type, up to 10 fold (Table 5).

Table 5: Comparative study of DPPH reducing power and iron-ferrous chelating power of hot, cold and ethanolic extracts, between the nine species of the Lamiaceae family.

PLANTS	IC_{50} DPPH (mg/ml)			IC_{50} FRAP (mg/ml)		
	MEAN HAE± Er.Std	MEAN CAE± Er.Std	MEAN EE± Er.Std	MEAN HAE± Er.Std	MEAN CAE± Er.Std	MEAN EE± Er.Std
C. officinalis	0,116±0,004 (b) (c)	0,335±0,004 (b)	0,217±0,008 (a)	0,2514±0,004 (a)	0,4347±0,008 (c)	0,2711±0,005 (a)
L. dentata	0,715±0,0007 (g)	0,379±0,003 (c)	0,235±0,0019 (a) (b)	0,972±0,002 (e)	0,7521±0,002 (e)	0,3465±0,004 (b)
M. pulgium	0,270±0,006 (e)	0,094±0,0007 (a)	0,245±0,008 (a) (b)	0,46056±0,008 (c)	0,3627±0,007 (b)	0,3521±0,009 (b)
M. routundifolia	0,133±0,008 (c) (d)	0,345±0,010 (b) (c)	0,255±0,011 (a) (b)	0,3324±0,009 (b)	0,3581±0,009 (b)	0,3344±0,005 (b)
O. compactum	0,075±0,001 (a)	0,075±0,001 (a)	0,246±0,012 (a) (b)	0,2454±0,011 (a)	0,2402±0,008 (a)	0,3422±0,009 (b)
O. majorana	0,160±0,008 (d)	0,348±0,007 (b) (c)	0,248±0,008 (a) (b)	0,3593±0,008 (b)	0,4459±0,009 (c)	0,3494±0,007 (b)
R. officinalis	0,096±0,001 (a) (b)	0,352±0,007 (b) (c)	0,264±0,007 (b)	0,256±0,008 (a)	0,4421±0,009 (c)	0,3251±0,006 (b)
S. officinalis	0,256±0,010 (e)	0,747±0,010 (e)	0,244±0,014 (a) (b)	0,559±0,011 (d)	0,8668±0,010 (f)	0,2625±0,009 (b)
T. satureioides	0,343±0,011 (f)	0,652±0,013 (d)	0,247±0,011 (a) (b)	0,4539±0,011 (c)	0,6394±0,014 (d)	0,3341±0,012 (a)
TOTAL	0,241±0,020	0,370±0,022	0,245±0,003	0,4322±0,023	0,5046±0,020	0,3241±0,004
FISHER	776,58 (p<0,000) **	808,12 (p<0,000) **	1,70 (p<0,111)	623,15 (p<0,000) **	463,27 (p<0,000) **	16,6 (p<0,000) **

Groups with the same letters do not differ significantly by tukey test; Er.Std: Standard Error; HAE: Hot Aqueous Extract; CAE: Cold Aqueous Extract; EE: Ethanolic Extract; **: very highly significant difference.

Chelation of metal ions is considered a very important antioxidant strategy in MAPs [44], because these metals can generate EOAs *in vitro* and *in vivo* [45], and contribute in triggering lipid peroxidation. Results of this study showed that the chelation of metal ions increased with increasing concentration of the extracts, this activity depended mainly on the type of solvent used for extraction. Indeed, it has been shown that polar solvents have a high capacity to chelate metal ions [40], which is in agreement with our results where HAE presented the strongest chelation power, followed by EE and CAE with significant differences between the 3 types of extracts (p<0.05). In addition, it is possible that the phenolic compounds extracted by maceration with hot water have supported that power in HAE, for which the dose-dependent relationship noticed implies that the high levels of phenolic compounds contributed to its chelating activity. Regarding EA and EAF, they may can be rich in aglycone and glycosylated flavonoids that do not have this chelating activity [46].

There was also a better antioxidant activity of the extracts in DPPH test compared to iron chelation test. In fact, the antioxidant activity is not only related to the composition of plants screened, but also to the revelation technique used for the same extract [47, 48]. DPPH assay is simple, fast and independentof the samples polarity [49], which may explain this hierarchy observed in antioxidant activity. The study of correlation between radical scavenging power and the capacity of metal ions chelation seems interesting, firstly

because oxidative stress is the cause of formation of free radicals through metals transition (iron, copper) via Fenton reaction [50]. Secondly, the evaluation of the plant antioxidant effect is made by combining the results of at least two *in vitro* assays [51].

The variables projection on the factorial plot allowed to study the correlation between the different parameters, their behaviors and characteristics of the species studied. According to this projection, we noted the presence of four groups. Variables of each group were correlated with each other (Figure 5). Groups 1 and 2 presented a similarity in evolution and showed a negative correlation to groups 3 and 4.

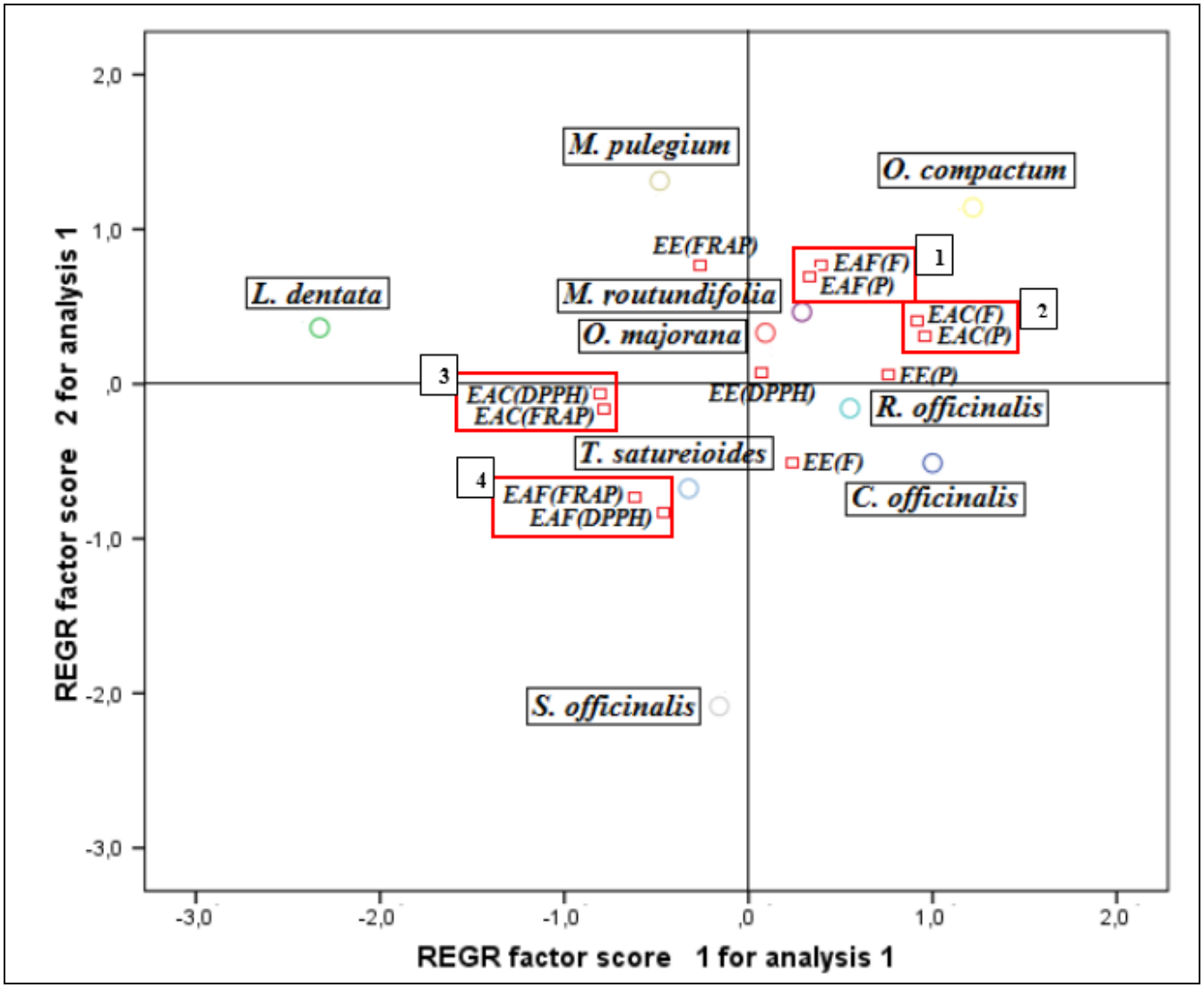

Fig 5: Variables projection of the Lamiaceae family. HAE: Hot Aquous Extract; CAE: Cold Aquous Extract; EE: Ethanolic Extract; DPPH: IC50 of DPPH test; FRAP: IC50 of FRAP test; P: Total Polyphenols; F: Flavonoïds.

As shown in figure 5, the IC_{50} values obtained from DPPH and FRAP assay were correlated for HAE (R^2 = 0.9648), which implied that antioxidants in this extract were able to scavenge free radicals and reduce oxidants. Furthermore, an average correlation was detected for the CAE (R^2 = 0.7077), indicating that it rather had the scavenger power of free radicals. This was in concordance with the results of Hua-Bin Li et *al.*, 2008 [52]. These results revealed that both the antioxidant evaluation methods were suitable, adequate and reliable for the same extract [53]. From the data obtained by two assay methods, it was found that almost all plants from the *LAMIACEAE* family, showed a high antioxidant capacity. These plants are thus valuable sources of natural antioxidants, both for preparation of crude extracts and for further isolation and purification of antioxidant components.

The correlation between their contents and those of the antioxidant activities have been extensively studied. Some authors have shown that there is no correlation between polyphenol content and free radical scavenging [54]. While others have shown that the presence of phenolic compounds, including flavonoids, phenolic acids, tannins can contribute to the MAPs scavenging effect of free radicals [50]. It was considered necessary to study the correlation between the polyphenols levels and the antioxidant activity.

The correlation coefficient between the polyphenols content of the HAE (R^2 = 0.857) and CAE (R^2 = 0.886) and the antioxidant activity was significant (p<0.05), indicating that 85% to 88% of the anti-oxidant capacity was due to the contribution of phenolic compounds which were the dominant antioxidants in these two extracts (Figure 5). These results were conforming to the results of some research groups that reported a positive correlation between total phenolic content and antioxidant activity [55, 39, 45, 56]. According to the same data, only extracts that were highly concentrated phenolic compounds were able to show an antioxidant capacity by scavenging DPPH radical. This was in accordance with

studies of Nguyen et *al.,* (2011) [40] performed on Vietnamese MAPs. On the other hand, it has been shown that phenolic compounds are the major constituents at the origin of the antioxidant activities of MAPs [32, 53, 52]. It is possible that the antioxidant activity was related to the nature of the substituted hydroxyl group, the number of aromatic rings, or the molecular weight of these molecules [57]. In addition, the synergy between the different phytochemicals should be considered in the biological activity [43].

Studies have recently shown that flavonoids have an antioxidant effect on human health. These compounds scavenge free radicals and chelate metal thus avoiding the Fenton reaction to occur [58]. As shown in figure 5, a strong significant correlation ($p<0.05$), with an inversely proportional relationship exist between flavonoids and DPPH radical scavenging for HAE ($R^2 = 0.8035$) and CAE ($R^2 = 0.8921$). A study on aqueous extracts of 11 Algerian MAPs has shown a good correlation with $R^2 = 0.79$ [41]. Many studies have attempted to elucidate the relationship between the chemical structure of flavonoids and their ability to scavenge free radicals (59). Indeed, it has been reported that the radical flavonoxy (FL-O •) react with another radical to form a stable quinone structure [60].

It seems that there was a moderate and significant correlation ($p<0.05$) between flavonoids content and chelation of ferrous iron (Figure 5), for both HAE ($R^2 = 0.7729$) and CAE ($R^2 = 0.7439$), with a chelating power of about 75%, suggesting that this capacity is not due to total flavonoid compounds extracted in this study. This was in extreme agreement with other studies, which reported that only certain classes of flavonoids with a hydroxyl group can chelate ferrous iron [58], and that isoflavones is the main class able to chelate *in vivo* and *in vitro* transition metals [61]. It would therefore be useful to investigate in these compounds via HPLC to elucidate their specific activities.

The principal component analysis (CPA) of the 9 *Lamiaceae* family species, showed that O. compactum, M. routundifolia, O. majorana, R. officinalis followed by C. officinalis, were characterized by the four groups, with high phenolic content and antioxidant power.

Thymus satureoides followed by S. officinalis were rather characterized by their EE which was rich in flavonoids and possessed a remarkable antioxidant effect.

Lavendula dentata has been poor in terms of chemical constituents analyzed, with the least perfoming antioxidant effect.

The level of correlation between the phenolic and flavonoid content and antioxidant activity is an interesting aspect, but it must be considered that the phenolic compounds respond differently in the analysis, depending on the number of phenolic groups and that total phenolic compounds may not incorporate all the antioxidants present in an extract [55]. This was the explanation that can be attributed to the antioxidant effect of the EE of this botanical family, while he shows no correlation vis-a-vis the polyphenol ($R^2 = 0.0849$) or flavonoid contents ($R^2 \leq 0.2$). While, a study on 11 EEs of Algerian MAPs showed a good correlation with $R^2 = 0.79$ [41].

2. Antiglycation activity

2.1 Electrophoretic analysis

Native-PAGE gel was used in order to evaluate the *in vitro* inhibitory effect on BSA glycation by the three types of extracts. The migration profiles were compared to the negative control (BSA non-glycated) and positive control (BSA-MG-metformin). The results are shown in table 5.

Tableau 5*:* Comparative study of migration distance on PAGE-Native gel in the presence of HAE, CAE and EE, between nine species from Lamiacae family.

PLANTS	MEAN I. HAE ± Er.Std			MEAN I. CAE ± Er.Std			MEAN I. EE ± Er.Std		
	A	B	C	A	B	C	A	B	C
C. officinalis	55,4±0,26 (b)	52,9±0,23 (b)	47,4±0,49 (b)	101,6±0,30 (c)	100,3±0,3 (d)	98,7±0,33 (d)	55,3±0,26 (b)	51±0,25 (b)	47±0,33 (c)
L. dentata	77,7±0,3 (f)	75,8±0,24 (g)	74,4±0,26 (h)	120±0,25 (g)	113±0,36 (e)	112,2±0,32 (e)	56±0,21 (b)	66,8±0,32 (d)	77,7±0,3 (i)
M. pulegium	70±0,29 (d) (e)	67,1±0,23 (e)	49,4±0,37 (c)	118,3±0,3 (f)	116,1±0,37 (f)	115,1±0,31 (f)	66,4±5,49 (c)	69,1±0,27 (e)	54,8±0,29 (d)
M. routundifolia	100±0,25 (g)	99,7±0,26 (h)	99,8±0,24 (i)	109,7±0,33 (d)	112,5±0,30 (e)	112,7±0,26 (e)	64,6±0,26 (c)	64,4±0,22 (c)	64,1±0,27 (f)
O. compactum	70,5±0,26 (e)	70,1±0,27 (f)	70,3±0,21 (g)	116±0,25 (e)	112,7±0,3 (e)	112,5±0,26 (e)	84,4±0,22 (d)	77,9±0,23 (f)	70,3±0,26 (g)
O. majorana	48,3±0,26 (a)	46,2±0,29 (a)	45,9±0,23 (a)	50,4±0,26 (a)	49,8±0,24 (a)	49,3±0,3 (a)	99,3±0,33 (e)	86,9±0,27 (g)	72,4±0,26 (h)
R. officinalis	68,8±0,32 (d)	62,2±0,24 (d)	56,8±0,32 (e)	72±0,33 (b)	62,2±0,38 (b)	54,5±0,37 (b)	70,8±0,24 (c)	66±0,29 (d)	61±0,25 (e)
S. officinalis	61,2±0,32 (c)	60,5±0,26 (c)	60,6±0,22 (f)	128,1±0,34 (h)	130,7±0,26 (g)	129,7±0,26 (g)	42,3±0,33 (a)	34,1±0,31 (a)	27,8±0,29 (a)
T. satureioides	60,7±0,3 (c)	60±0,21 (c)	55,2±0,24 (d)	130±0,25 (i)	98,2±0,87 (c)	64,7±0,26 (c)	139,3±0,21 (f)	138,1±0,31 (h)	32±0,36 (b)
TOTAL	68,066±1,49	66,055±1,53	62,2±1,71	105,12±2,68	99,5±2,65	94,37±2,99	75,378±2,97	72,7±2,87	56,34±1,76
FISHER	2627,94 (p<0,000) **	3680,87 (p<0,000) **	3165,74 (p<0,000) **	8146,71 (p<0,000) **	3950,64 (p<0,000) **	9824,69 (p<0,000) **	10404,79 (p<0,000) **	10404,79 (p<0,000) **	3562,45 (p<0,000) **

Groups with the same letters do not differ significantly by tukey test; Er.Std: Standard Error; HAE: Hot Aqueous Extract; CAE: Cold Aqueous Extract; EE: Ethanolic Extract; I. Fluorescence Intensity; A : 1.5 mg/ml ; B : 3.5 mg/ml ; C : 10 mg/ml ; **: very highly significant difference.

Electrophoretic test showed that, in the case of EE, all the plants tested presented a dose-dependent anti-glycation effect with migration distances, in the concentration of 10 mg/ml, ranging from 78 mm in *L. dentata* to 28 mm in *S. officinalis,* with the exception of *T. satureioides*, which showed its activity only in the highest concentration (10 mg/ml).

For the HAE, almost all plants have a dose-dependent anti-glycation effect. the maximum activity was recorded in *O.*

majorana at 10 mg/ml with a migration distance of 15 mm from the band of BSA non-glycated. While, *M. rotundifolia* showed no inhibitory effect on glycation, because the migration of bands was parallel to that of control band (BSA glycated). Concerning the CAE, anti-glycation effect was generally small compared to that of EE and HAE, with a noticed absence of inhibition in the case of *S. officinalis*.

According to Kanska U. and Boratynski J., 2002 [62], the acidity could affect the migration of glycated BSA. Therefore, to ensure that the recorded inhibitory effect for some extracts is attributed to bioactive molecules and not to the acidity, the measure of pH was performed and revealed that they vary between 5.37 and 7.26 (n = 3) (table 2). So, the effect observed previously was due to the active substance of these plants rather than their acidity that is closely related to their biochemical composition. In fact, a study has shown that the acidity of a plant extract could be related to parameters such as partial fermentation of samples due to the drying time, and the enzymatic activity during initial phase of drying [63].

It has been reported that the MG is an intermediate in the synthesis of AGEs. Indeed, it reacts with plasma proteins and causes there *in vitro* modifications under physiological conditions. Due to its abundance in serum, BSA is a protein which undergoes glycation on multiple sites [64]. BSA-MG link is already described in the literature [65].

Non glycated BSA has a molecular weight of 66.65 KDa [64]. The binding of this protein to MG reduce this weight, which takes away the positive charge to the complex [66]. Thus, it migrates farther from the band of nonglycated BSA. These data are consistent with our results where extracts that not had any anti-glycation activity migrated farther or in the same level to the band of nonglycated BSA. The migration of extracts with high antiglycation activity has slowed, and their bands were very close to that of metformin. This means that there was an inhibition in the first steps of the glycation process, during formation of AMADORI products. In general, inhibition of glycation may be on several levels: on the one hand, the cleavage of cross-linking products and blocking AGE receptors, on the other hand, the chelation of transition metals and the free radical scavenging, or the antidiabetic therapy in order to reduce hyperglycemia [67].

2.2 Fluorimetric analysis

The inhibitory power of *in vitro* formation of fluorescent AGEs was investigated by the BSA-MG system. The fluorescence intensity of our extracts was compared with that of MG-BSA complex without inhibitor. The results are shown in table 6 and figure 6.

Table 6: Comparative study of fluorescence intensities in the presence of HAE, CAE and EE, between nine species from Lamiacae family.

PLANTS	MEAN I. HAE ± Er.Std			MEAN I. CAE ± Er.Std			MEAN I. EE ± Er.Std		
	A	B	C	A	B	C	A	B	C
C. officinalis	465,93±0,95 (f)	402,54±0,89 (e)	213,58±0,59 (b)	475,07±0,87 (e)	430,35±0,16 (e)	300,76±0,09 (e)	422,27±0,11 (e)	360,65±0,09 (b)	254,52±0,12 (c)
L. dentata	335,51±0,12 (b)	306,26±0,18 (b)	238,33±0,07 (c)	372,44±0,17 (b)	332,25±0,12 (b)	262,02±0,07 (c)	398,546±0,15 (c)	392,00±0,12 (e)	400,7±0,22 (g)
M. pulegium	532,31±0,13 (h)	486,69±0,16 (h)	297,20±0,17 (f)	555,10±0,27 (g)	590,35±0,16 (i)	479,15±0,11 (i)	534,588±0,17 (h)	565,35±0,25 (h)	318,52±0,14 (e)
M. routundifolia	396,47±0,16 (d)	370,22±0,19 (d)	346,70±0,12 (h)	412,797±0,18 (c)	409,55±0,97 (c)	353,09±0,24 (g)	470,378±0,15 (f)	429,84±0,06 (g)	449,30±0,12 (h)
O. compactum	551,26±0,12 (i)	457,37±0,13 (g)	389,52±0,22	440,73±0,18 (d)	471,60±0,15 (f)	302,31±0,14 (f)	488,145±0,16 (g)	418,33±0,15 (f)	298,52±0,16 (d)
O. majorana	270,50±0,12 (a)	223,44±0,13 (a)	283,24±0,15 (e)	286,21±0,14 (a)	297,34±0,16 (a)	287,44±0,12 (d)	214,538±0,15 (a)	377,54±0,14 (c)	355,34±0,15 (f)
R. officinalis	492,54±0,16 (g)	415,42±0,18 (f)	243,55±0,14 (d)	516,74±0,08 (f)	416,49±0,13 (d)	240,68±0,15 (b)	578,407±0,16 (i)	581,42±0,16 (h)	539,42±0,13 (i)
S. officinalis	451,48±0,13 (e)	327,59±0,17 (c)	319,47±0,16 (g)	575,35±0,16 (h)	497,48±0,15 (g)	443,56±0,26 (h)	413,225±0,11 (d)	385,48±0,13 (d)	254,46±0,15 (b)
T. satureioides	375,40±0,17 (c)	300,3±0,14 (b)	165,26±0,15 (a)	710,24±0,11 (i)	515,56±0,14 (h)	183,49±0,13 (a)	261,387±0,14 (b)	187,46±0,13 (a)	8,45±0,12 (a)
Total	430,15±9,30	366,31±8,32	277,43±6,96	482,74±12,45	440,11±9,08	316,94±9,45	420,16±11,89	410,90±11,60	319,91±14,93
FISHER	71998,02 (p<0,000) **	61539,13 (p<0,000) **	78248,41 (p<0,000) **	137868,89 (p<0,000) **	65450,41 (p<0,000) **	345017,19 (p<0,000) **	627761,21 (p<0,000) **	594232,96 (p<0,000) **	935252,92 (p<0,000) **

Groups with the same letters do not differ significantly by tukey test; Er.Std: Standard Error; HAE: Hot Aqueous Extract; CAE: Cold Aqueous Extract; EE: Ethanolic Extract; I. Fluorescence Intensity; A : 1.5 mg/ml ; B : 3.5 mg/ml ; C : 10 mg/ml ; **: very highly significant difference.

From these data, it was clear that the inhibitory power varied from a plant to another within the same family, but also according to the type of extract. But in general, the HAE followed by EE have given the best inhibitory activities, which was in extreme agreement with the study of Harris CS et *al.,* (2011) [68], who obtained a strong inhibitory effects from the EE of 17 Canadian MAPs. It was also found that all plants showed a dose-response inhibitory effect on the *in vitro* glycation, with a highest inhibitory capacity registered in *T. satureioides* in all types of extracts, followed by *A. officinalis* and *L. dentata* for CAE, *C. officinalis* and *L. dentata* for the HAE.

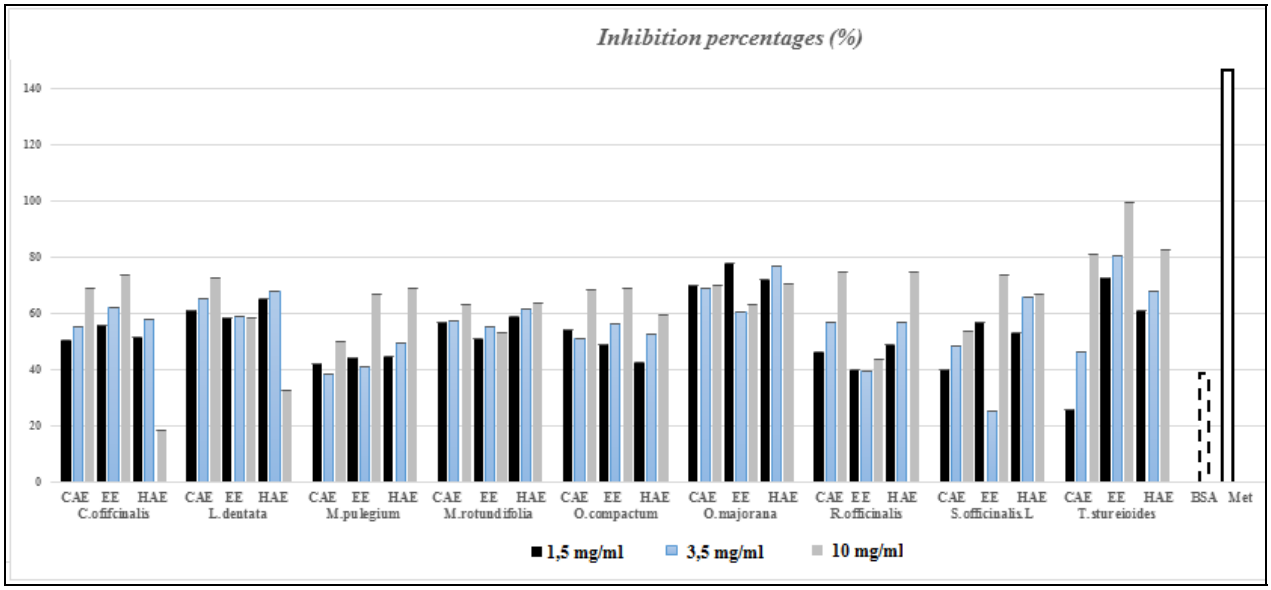

Fig 6: Inhibition percentage of glycation by CAE, EE and HAE. Met : control + ; BSA : control-, BSA-MG System and Metformin.

Indeed, the decrease in the fluorescence intensity indicates an inhibition of AGEs formation, which are formed during the last irreversible steps of glycation. Thus, plant extracts of the Lamiaceae family possess strong anti-glycation properties which act simultaneously during the first and last stages of the glycation process.

The dendrogram was used to classify the species into several groups and plot a tree diagram, taking into consideration the two parameters studied previously: electrophoresis analysis and fluorometric analysis (Figure 7).

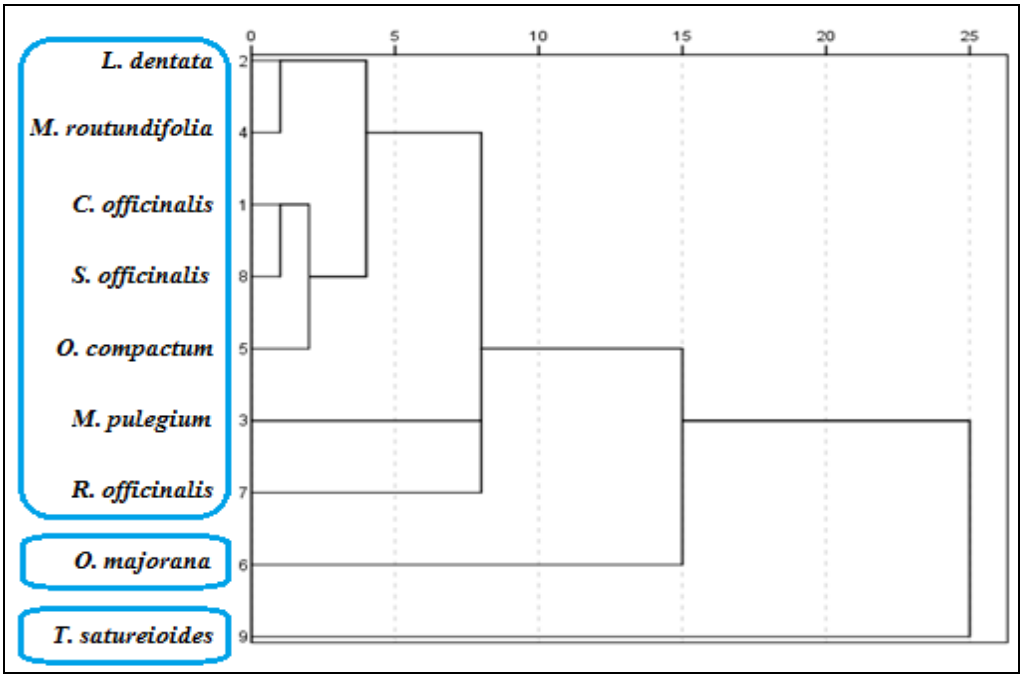

Fig 7: Tree diagram using the average distance between the nine species of the Lamiaceae family.

According to the dendrogram of figure 7 and on the basis of the distance of classes combination, it appeared that there were three main groups of vegetable species. The group with the best anti-glycation power was that represented by *T. satureiodes*, followed by that of *O. majorana*. Whereas the third group showed the least antiglycation effect.

The results of research showed that glycation acts synergistically with oxidative stress generating diabetes complications. In addition, several studies have shown a relationship between antioxidant activity and anti-glycation MAPs extracts [37]. In fact, it has been demonstrated the ability to inhibit glycation induced by methylglyoxal and glyoxal by antioxidants [69]. Thus, extracts of plants belonging to the *Lamiaceae* family exhibiting a high anti-oxidation and anti-glycation activity and high levels of polyphenols and flavonoids, would present major, safe and effective targets in the anti-diabetes therapy. However, a study performed on EEs of Mauritanian MAPs has shown that there is practically no relationship between anti-oxidation and anti-glycation activity [70], which was reflected in our study with zero correlation between these two activities. These results suggest the idea that anti-oxidation activity is not always accompanied by anti-glycation activity. This observation led us to conclude that other mechanisms are involved in anti-glycation activity of these extracts.

The correlation study showed a very low coefficient R^2 between total polyphenols contents and the inhibitory effect of glycation. In addition, this effect is not affected by the

reducing sugars content of extracts ($R^2 \leq 0{,}002$). It is highly probable that the anti-glycation activity of the extracts on average antioxidant activity and low levels of polyphenols and flavonoids is related to other bioactive compounds extracted from these plants.

According to recent studies, saponins were the compounds with the most remarkable anti-glycation effect compared to polyphenols and flavonoids. These substances are hypoglycaemic agents, and their mode of action is not well known [71, 72]. Also, anthocyanins are described as efficient inhibitors of glycation [73]. An investigation on 10 metanolic extract from the *Lamiaceae* family wich included *R. officinalis*, *L. dentata* and *O. majorana*, chowed that the Rosmarinic acid turned out to be the most predominant phenolic compound in all plant organs examined, and have a possible role as the chemotaxonomic marker of this family [74]. The same study showed that rosmarinic acid was accompanied by other hydroxycinnamic (caffeic acid) or benzoic (protocatechuic, *p*-hydroxybenzoic and gentisic acids) derivatives. Indeed, rosmarinic acid is known as an antiviral, antibacterial, antioxidant, anti-inflammatory and immunostimulating agent [75-77].

The presence of alkaloids, flavonoids and tannins in the different species analyzed is an important indicator for their hypoglycemic or antidiabetic activity. It is demonstrated by several studies that these secondary metabolites would have this activity [78-81]. Several mechanisms are attributed to flavonoids for this activity. According to several studies, flavonoids prevent diabetes by inhibiting reductase alkalosis [78, 79]. In addition, eating foods rich in flavonoids is inversely correlated with the risk of developing cardiovascular disease [60]. Moreover, the anti-diabetic action of tannins is due to its action on the diabetes itself at the cellular level by promoting the action of insulin and on diabetes complications by their antioxidant and anti-enzymatic power, neutralizing the effect of free radicals and limiting the inflammatory response in different tissues. According to other studies, the significant and enduring hypoglycemic effect could be related to the presence of both flavonoids and alkaloids compounds that probably act synergistically [80].

Conclusion

This study has shown that plants of the *Lamiaceae* family are promising sources of natural antioxidants with significant levels of polyphenols and flavonoids in the three types of extracts. Moreover, they present interesting anti-oxidation and anti-glycation activities. *In vitro* evaluation of anti-glycation activity revealed that it is not necessarily accompanied by an antioxidant activity. Indeed, some extracts such as HAE of *L. dentata* and CAE of *T. satureioides* have shown strong anti-glycation effect while their antioxidant effect remains poor. According to the results obtained, the ethanol extract concentrated better the active compounds. Furthermore, these results seem to be important from the point of view of additional pharmacological applications of medicinal plants belonging to the *Lamiaceae* family. These results can be exploited for purification of the active principles and preparation of improved forms of effective herbal remedies from *T. satureioides*. Current therapy targeting the production of AGEs has encountered many failures, especially with the side effects associated with synthetic glycation inhibitors. The intervention of active substances from our extracts may present an interesting therapeutic approach in reducing diabetic complications AGEs and free radicals.

Conflict Of Interest

The authors declare that there is no conflict of interest.

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