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Study on antioxidant and hypolipidemic effects of polyphenol-rich extracts from *Thymus vulgaris* and *Lavendula multifida*

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ABSTRACT

In the present study, the polyphenol-rich extracts of two medicinal plants widely used in Errachidia country (south east of Morocco) (*Thymus vulgaris* and *Lavendula multifida*) were assessed for their antioxidant, hypocholesterolaemic and hypotriglyceridaemic activities.The antioxidant activity of polyphenol-rich extracts was assessed by using the FRAP assay (Ferric Reducing Antioxidant Power), the RSA method (Radical Scavenging Activity) and the inhibition of the AAPH (2, *2*'-azobis (2-amidinopropane) hydrochloride)-induced oxidative erythrocyte hemolysis. Hyperlipidemia was induced in rats by intraperitoneal injection of Triton WR-1339 at a dose of 200 mg/kg body weight. The animals were divided into normolipidemic control group (NCG), hyperlipidaemic control group (HCG) and hyperlipidaemic plus herb extracts (0.2 g/100 g body weight). However, 24 h after treatment by polyphenol-rich extract of *Thymus vulgaris* and *lavendula multifida* we not detect any significant effect on both plasma total cholesterol and triglycerides profiles. Our results indicate that, the aqueous extract from *lavandula multifida* and T*hymus vulgaris,* present a higher antioxydant activities. Indeed, *Lavandula multifida* presents an anti-hemolysis activity equivalent to that exhibited by *Thymus vulgaris.* The addition of AAPH decrease the half time of hemolysis by 45%. The polyphenol- rich extracts from *thymus vulgaris* and *lavendula multifida* varieties increase the half time hemolysis by 533% and 479%, respectively. Although, these two varieties of thyme and lavender did cause any hypolipidemic activity. The results found are encouraging for further assessment to elucidate the mechanism of action and to identify the bioactive compounds implicated in the antioxidant effect and the membrane stability.

Keyswords: Antioxidant effect; Hypolipidemia; Polyphenol; Erythrocyte hemolysis; *Thymus vulgaris*; *Lavandula multifida*.

INTRODUCTION

Oxidative stress is believed to be a primary factor in various diseases as well as in the normal process of aging (1,2). Free radicals and reactive oxygen species (ROS) are well known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, atherosclerosis and cardiovascular diseases (3,4). Cardiovascular diseases are the most common cause of death in the industrialized countries (5). Many epidemiological and experimental studies have shown that the polyphenol intake is inversely correlated with atherosclerosis development and related cardiovascular events (6-9). The beneficial effect of polyphenols is associated with a multitude of biological activities, including antioxidant and free radical-scavenging properties, anti-platelet aggregation and inhibition of vascular smooth muscle cell proliferation, all these effects might interfere with atherosclerotic plaque development and stability. These observations might explain their cardio-vascular protective properties (1`0). On the other hand, it is now established that

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hyperlipidaemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications (11). A logical strategy to prevent or treat atherosclerosis and reduce the incidence of cardiovascular disease events is to target the hyperlipidaemia and oxidative stress by diet and/or drug intervention.

Lavender and thyme are largely used in the Moroccan folk medicine. In fact, *Lavandula multifida* L. is traditionally used to treat headaches, depression, diabetes, and for their sedative properties (12,13). It is used also to obtain lavender essential oil, rich in monoterpenes and employed for its antimicrobial and carminative properties to treat burns and for cosmetic purposes (14). Its leaves and stems are used in the Moroccan folk medicine to prepare decoctions against rheumatism, chill and as digestive system benefices agent (15). Furthermore, an inflammatory activity is revealed in *lavandula multifida* (16). *Thymus vulgaris* is commonly used in folk medicine as an antiseptic, bronchial and spasmolytic agent. The herb is used internally in upper respiratory tract disorders and externally in skin disorders. *Thymus vulgaris* is quoted by various authors for its polyphenol and flavonoid contents and its antioxidant, anti-inflammatory, vasorelaxant and antispasmodic activities (17). Thyme is also capable to induct a prolongation of the lag-time in the LDL oxidation assay (18).The aim of the present study is to examine the antioxidant and hypolipemic effects of the polyphenol rich extracts of *Lavandula multifida* L and *Thymus vulgaris.*

MATERIAL AND METHODS

Plant material

Lavandula multifida was collected in April-Mai 2007 in the Errachidia region, Morocco. The plants were identified by Dr. Ibn Tatou and a voucher specimen was deposited at the herbarium of the Scientific Institute, Université Mohammed V, Rabat, Morocco (N° : RAB 77497).*Thymus vulgaris* was cultivated in the botanic garden of Faculty of Sciences and Technologies, Errachidia Morocco

Preparation of plants extracts

The aqueous extracts were prepared using a manner similar to that used by patients with some modifications. The dried aereal parts of the herbs were decocted 30 min in distilled water (100 °C), filtered and the obtained solution was concentrated in rotatory evaporator under vacuum at 65 °C.

Determination of total phenol contents

The polyphenol content in aqueous extracts was determined according to the Folin–Ciocalteu colorimetric method (19). cafeic acid was used to make the calibration curve. The result (total phenols) was expressed in mg per g of cafeic acid equivalents (mg/g extract) measurements were done in triplicate.

Determination of flavonoids contents

The flavonoids content in extract was determined spectrophotometrically according to Jay et al. (20) using a method based on the formation of a complex flavonoid-aluminium, having the maximum absorbance at 430 nm. Rutin was used to make the calibration curve. The flavonoids content was expressed in mg per g of rutin equivalent (RE) (mg/g extract). The analyses were done in triplicate

Antioxidant study of thyme and lavender polyphenol-rich extracts

RSA assay (DPPH Radical Scavenging Activity)

DPPH (1,1-diphenyl-2-picryl-hydrazil) is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The model of scavenging the stable DPPH radical is widely used for relatively rapid evaluation of antioxidant activities compared to other methods (21). The reduction capability of the DPPH radical is determined by its absorbance decrease at 517 nm, as induced by natural antioxidants. An aqueous solution of the sample was added to an ethanolic solution of DPPH according to the method of Barros et al. (22). Three concentrations were prepared for each sample. After mixing gently and leaving to stand for 30 min at room temperature, the absorbance was read at 517 nm and Trolox was used as DPPH-scavenging positive control compound. The results are expressed as inhibitory concentration 50 (IC50).

FRAP assay (Ferric Reducing-Antioxidant Power)

The method is based on the reduction of the Fe^{3+}TPTZ (tripyridyl triazine) complex to the ferrous form at low pH (23). This reduction is monitored by measuring at 37°C, the absorption change at 593 nm under in acid conditions (pH 3,6).

Inhibition of the AAPH-induced erythrocyte oxidative haemolysis

The antioxidant activity of the plant extracts was measured as the inhibition of the AAPH-induced oxidative erythrocyte hemolysis according to the procedure established by Prost (24) with slight modifications. Blood was obtained from a rabbit and diluted with heparined 10 mM phosphate buffer saline (PBS) at pH 7.4. In order to induce free radical chain oxidation in the erythrocytes, aqueous peroxyl radicals were generated by thermal decomposition of AAPH (2,2,-azobis(2-amidinopropane hydrochloride) in

oxygen (25). A rabbit erythrocyte suspension in PBS was used to make different samples:

- Control sample: Erythrocyte suspension in PBS;
- Haemolysis sample: Erythrocyte suspension in PBS+AAPH (2,2,-azobis(2-amidinopropane hydrochloride);
- Plants extract samples : Erythrocyte suspension in PBS +AAPH + plant extract (0,143 mg/ml).

The reaction mixture was shaken gently and incubated at 37 °C. The absorbance was read at 540 nm. The optical density is read every 5 minutes, with the aim of measuring most correctly possible the time of half-haemolysis. The half-time of haemolysis corresponds in necessary time so that the initial optical density decreases in 50 %. It corresponds to 50 % of haemolysis of the initial erythrocytes. A high half-time of haemolysis corresponds to a good resistance of erythrocytes. The addition of an antiradical substance will lead to an increase of the half-time of haemolysis.

Hypolipidemic study of thyme and lavender polyphenol-rich extracts

Animals and treatment

Adult female Wistar rats weighing 170–200 g bred in the animal house of the department of Biology (Faculty of Sciences, Oujda, Morocco) were housed in a controlled room with a 12 h

light-dark cycle, at room temperature of 22±02 °C, and kept on standard pellet diet (Société SONABETAIL, Oujda, Morocco). Animal maintenance and handling were in accordance to internationally accepted standard guidelines for use of laboratory animals.

Experimental animal protocol

Overnight fast rats were divided into seven groups of sex rats each. The first group served as normolipidemic control (NCG), received intraperitoneal administration of normal saline and water by gavage, the second, hyperlipidaemic control group (HCG), was treated with intraperitoneal injection of Triton WR-1339 (Tyloxapol, Sigma–Aldrich, USA) at a dose of 200 mg/kg in normal saline and gavaged with distilled water; in the plant treated groups, the animals were also treated with intraperitoneal injection of Triton (200 mg/kg BW) followed by administration of aqueous extracts from *Thymus vulgaris* and *Lavendula multifida* by gavage. In the following period of study (24 h), animals have access only to tap water. After 24 h from treatments, animals were anaesthetized with diethyl ether and blood was taken from their tail vein using heparinized capillary. The blood samples were immediately centrifuged (2500 rpm/10 min) and plasma used for lipid analysis.

Biochemical analysis of plasma

Triglycerides in plasma were quantified by an enzymatic method using Bio Sud Diagnostici kits (Bio sud Diagnostici S.r.l. Italy). Briefly, after enzymatic hydrolysis with lipases, the formation of quinoneimine from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic effect of peroxidise, was followed spectrophotometrically at 540 nm.

Total cholesterol levels were determined by the cholesterol oxidase enzymatic method, using Biosud Diagnostici Kits (Bio Sud Diagnostici S.r.l Italy); cholesterol was hydrolyzed and, in the presence of phenol, the quinoneimine as indicator was formed from hydrogen peroxide and 4-aminoantipyrine via peroxidase catalysis and spectrophotometrically measured at 510 nm.

HDL-cholesterol concentrations were quantified by the same method as used to determine total cholesterol after removal of other lipoproteins by precipitation with phosphotungstic

acid (PTA) and $MgCl_2$ (Sigma Diagnostic kit, Inc, USA).

LDL-cholesterol was calculated by the Friedwald formula (26): LDL-Cholesterol = total cholesterol – (HDL-Cholesterol + TG/5)

Statistical analysis

Data obtained were analyzed using the Student's t-test and a P value less than 0,05 was considered statistically significant. Our results are expressed as means ± SEM.

RESULTS

Polyphenol content of Thymus vulgaris and Lavendula multifida extracts

The total polyphenols and flavonoids contents of *Thymus vulgaris* and *Lavendula multifida* extracts are shown in Table 1. The total polyphenols content is 356±9,79 mg eq cafeic acid/g of *Thymus vulgaris* extract and 199,16 ±11,20 mg eq cafeic acid/g of *Lavendula multifida* extract. The flavonoids content is 186,93 ±25,19 mg eq rutin/g of *Thymus vulgaris* extract and 142,55 ±1,66 mg eq rutin/g of *Lavendula multifida* extract.

Antioxidant activities of thyme and lavender varieties

The antioxidant activities of thyme and lavender varieties are shown in Table 1. Using the RSA method the radical scavenging activity from *Thymus vulgaris* IC50 is 0,7 ±0,02 mg/ml and from *lavendula multifida* IC50 is 2,6 ±0,01 mg/ml. The use of FRAP assay shows that the antioxidant activity from *Thymus vulgaris* is

48,03 ±0,66 mmol trolox/g and 12,76 ±0,48 mmol trolox/g from *lavendula multifida*.

The inhibitions of the AAPH induced erythrocyte oxidative haemolysis by the polyphenol-rich extracts from the two varieties are shown in Table 2. The addition of AAPH decreases the half time of haemolysis by 45%. While, the polyphenol- rich extracts from *Thymus vulgaris* and *Lavendula multifida* varieties to the erythrocytes suspension with AAPH increase the half time of haemolysis by 479 and 533%, respectively.

Effect of Triton and plant extracts on plasma lipid profile

The plasma total cholesterol and triglyceride levels of all groups at 24 h after treatments are shown in Fig1. In comparison with the normal control group (NCG), Triton WR-1339 caused a marked increase of plasma total cholesterol and triglyceride levels in hyperlipidemic control group (HCG). In fact, 24 h after Triton administration the increase of plasma total cholesterol concentration was of 391 % in HCG with respect to the NCG. Triglycerides levels were also elevated by 789 % in HCG. HDL and LDL-cholesterol concentrations are shown in Fig2. The HDL-cholesterol was not significantly changed in HCG with respect to its relative control group (NCG). LDL-cholesterol levels were elevated by more than 11 times in HCG than in normal control grouped animals.After 24h treatment, the administration of aqueous *Thymus vulgaris* or *lavendula multifida* extracts to Triton injected rats did exert any significant effect on all plasma lipid parameters (Fig 1 and Fig 2).

DISCUSSION

Triton WR-1339 has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidaemia in experimental animals (27,28). Many authors used this modele for a number of different aims (29,30) including studies of lipid metabolism and screening natural and chemical hypolipidaemic drugs (25). Many plants such as *Vaccinum myrtillus* (31), *Phyllanthus niruri* (32) have been investigated for their acute hypolipidaemic activity by the use the Triton WR-1339 to induce hyperlipidaemic animals. In our hand, the same model gave similar pattern of lipid profile changes at 24 h after Triton injection and demonstrates the feasibility of using it to assess the acute hypolipemic activity of aqueous thyme and lavender extracts. Present results show that aqueous *Thymus vulgaris and lavendula Mmultifida* exert no significant effect on both plasma cholesterol and triglycerides levels at 24 h from treatment.

Thymus vulgaris is quoted by various authors for its polyphenol and flavonoids contents and its antioxidant activity (17). Compared to T*hymus vulgaris, lavandula multifida* present a lesser polyphenol and flavonoids content and Ferric reducing /antioxydant activities. On the other hand, using the RSA method, the *lavandula multifida* polyphenol-rich extract presents a radical scavenging activity (IC50 = 2,6 ±0,01 mg/ml) exceeding that measured on the level of *thymus vulgaris (*IC50 = 0,7 ±0,02 mg/ml).

The thymus polyphenol-rich extract presents a strong antioxidant activity as demonstrated by both FRAP and RSA tests. In haemolysis test this extract is able to neutralize the free radicals liberated by the AAPH. This antioxidant activity protects the erythrocyte membrane from lesions and lead to an increase of the half-time haemolysis. The antioxidant activity of this extract can be linked up to the high polyphenols and flavonoids content. Divers studies mentioned an implication of the polyphenols and flavonoids in the antioxidant activity of different plants extracts (33,34). Phenolics have been shown to possess an important antioxidant activity toward these radicals, which is principally based on the redox properties of their phenolic hydroxyl groups and the structural relationships between different parts of their chemical structure (35,36). It have been established a highly positive relationship between total phenols and antioxidant activity in many plant species (37). We also note that *Lavandula multifida* although it posts only one weak antioxydant capacities; it however, presents an anti-haemolysis activity equivalent to that exhibited by *Thymus vulgaris*. Moreover, the addition of the thymus or lavender aqueous extract in haemolysis test inducts an increase of the half-time haemolysis which is superior to that showed by the witness. This indicates an increase of the level of erythrocytes membranes stability. The action of the thymus or lavender aqueous extract is not limited to inhibit the free radicals, but it also seems to have an influence on the structural stability of the erythrocyte membrane.

Biological membranes can be affected by many natural products present in medicinal plants (38). Various authors mentioned that flavonoids, the widely distributed subgroup of the polyphenol, have beneficial effect on the erythrocyte membrane stability (17,39,40).

Flavonoids can be incorporated into the erythrocyte membranes (39). Furthrmore,De Freitas et al. (40) relate that the exacerbation of the van der Waals

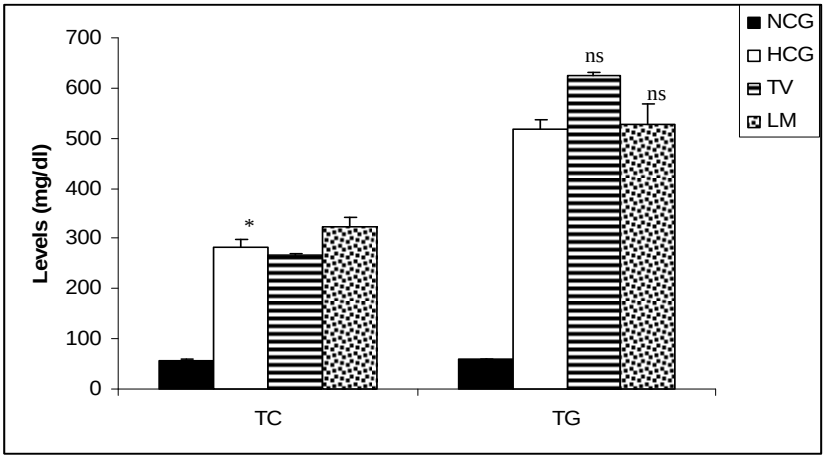

Fig. 1: Effect of Thymus vulgaris and Lavandula multifida on rat plasma total cholesterol and triglycerides
TC: total cholesterol; TG: triglycerides; HCG: hyperlipidelic control group; NCG: normolipidemic control group; TV: thyme treated group; LM: lavender treated group.
*Data are expressed as mean ± SEM ; *P<0,001; ns: not significant (HCG versus NCG; TV and LM versus HCG)*

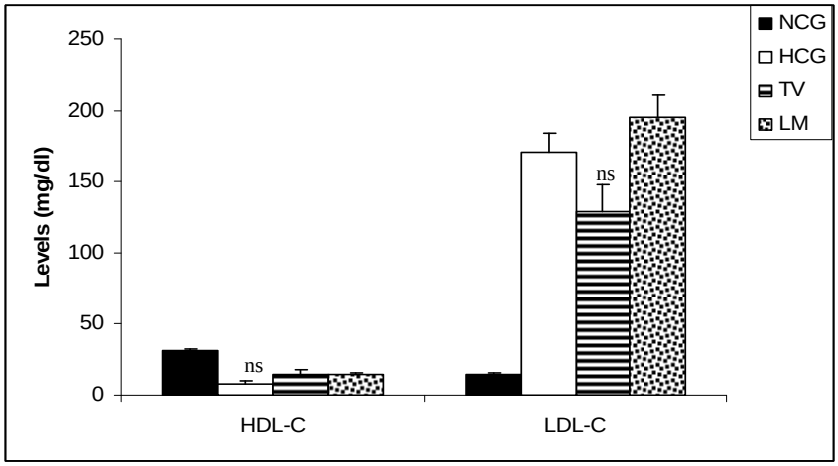

Fig 2: Effect of Thymus vulgaris and Lavandula multifida on plasma HDL and LDL-cholesterol in rats
HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; HCG: hyperlipidelic control group; NCG: normolipidemic control group; TV: thyme treated group; LM: lavender treated group.
Data are expressed as mean ± SEM
**P<0,001; ns: not significant (HCG versus NCG; TV and LM versus HCG)*

Table 1: Polyphenol content and antioxidant activity of Thymus vulgaris and Lavandula multifida polyphenol-rich extract.

	Thymus vulgaris	***Lavandula multifida***
Yield dry extract (%)	14.8	6.4
FRAP (mmol trolox/g PRE)	48.03±0.66	12.76±0.48
RSA (IC50 / mg/ml PRE)	0.7±0.02	2.6±0,01
Polyphenols (mg eq ac caféique/ g PRE)	356±9.79	199.16±11.20
Flavonoids (mg eq de rutin/g PRE)	186.93±25.19	142.55±1.66

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Table 2: Antihemolytic activity of aqueous thyme and lavender extracts.

	Haemolysis half-time (min)	% diviation
Control	073.33 ± 2.88	
AAPH sample	040.00 ± 0.01*	- 45 %
AAPH + Thymus vulgaris	253.33 ± 5.77*	+ 533 %
AAPH + Lavendula multifida	231.66 ±2.88*	+ 479 %

*P<0.001 (AAPH versus control ; *Thumus vulgaris* and *Lavendula multifida* versus AAPH)

contacts inside the lipid bilayer by the flavonoids could be a source of membrane stabilization.

A good part of the antioxidant activity and consequently the resistance of the erythrocytes to hemolysis inducted by the aqueous extract of *Thymus vulgaris* or *Lavendula multifida* varieties can be linked up to the content of polyphenols and flavonoids.

Although, *Lavandula multifida* and *thymus vulgaris* varieties don't post any hypolipemic activities. The fact that they exert a positive interaction on the stability of the cellular membranes, these two varieties deserves a more deeps study to bring their anti- atherosclerosis power.

CONCLUSION

Our results indicate that, compared to T*hymus vulgaris,* the aqueous extract from *lavandula multifida* present a lesser polyphenol and antioxydant activities. However, it presents an anti-hemolysis activity equivalent to that exhibited by *Thymus vulgaris.* In more, the aqueous extract from these thyme and lavender varieties contribute to the improvement of the erythrocyte membranes stability. These two varieties of thyme and lavender did not post a hypolipedimic activity. However, the fact that they exert a positive interaction on the stability of the cellular membranes opens applications to the anti atherosclerotic process level. The results found are encouraging for further assessment to elucidate the mechanism of action and to identify the bioactive compounds implicated in the antioxidant effect and membrane stability.

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