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Phytochemical evaluation and vasodilatory activity of O. elongatum, C. salviifolius and C. laurifolius

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Abstract

The high blood pressure is one of the main causes of death and cardiovascular diseases. Vasodilator medications are frequently used to treat arterial hypertension. The aim of this study was to evaluate vasodilator properties of three plants from North of Morocco then to quantify phenolic compounds and flavonoids in each plant extracts. The leaves of Origanum elongatum, Cistus salviifolius and Cistus laurifolius were extracted with hexane, methanol and ethyl acetate by ultrasonic apparatus. The total phenolic content, analyzed using Folin–Ciocalteu's reagent, of the samples varied from 46.6 µg/100 mg to 153 µg/100 mg dry weight, expressed as gallic acid equivalents (GAE). The total flavonoid concentrations, detected using 2% aluminum chloride, varied from 3.63 to 5.54 µg equivalents (RE)/mg dry weight.

Under different plant growth regulators induce a vasodilator effect on Wistar rat mesenteric vascular bed pre-contracted with norepinephrine. for O.elongatum, the methanolic extracts at (PP = 50 mmHg) were found to be more active than the acetylcholine with a concentration of 10^{-4} M taken as a reference (PP = 40 mmHg) and The vasodilator activity of methanolic extract of Cistus laurifolius at 100 µg/ml is the same as the reference (PP=40 mmHg). the vasodilator activity found in the three plants studied is due to their antioxidant power.

Keywords: Origanum elongatum, Cistus salviifolius and Cistus laurifolius, vasodilator activity

1. INTRODUCTION

Cardiovascular diseases are considered the main mortality cause in the world. Hypertension is characterized by blood pressure increase, related to heart effort to propel blood and the vascular resistance **[1].** This disease affects people from different ages and social ranges, and there are several attempts to minimize or prevent hypertension occurrence, in their majority, involve adequate diet, physical exercises, and medications. Burn et al, have demonstrated a close relationship between the antioxidant activity and the vasodilator capacity of a group of phenolic compounds **[2].** Similarly, other studies have concluded that antioxidants act as vasodilators, therefore, it helps to lower blood pressure and prevent vascular cerebral stroke **[3,4].** It is well known that the North of Morocco assumed to be rich of medicinal plant, they present important charges of phenolic and flavonoid contents, and possess significant antioxidant effect **[5,6],** O. elongatum extract exhibits the highest antioxidant activity. This finding was proving by Oualili et al **[7].** thanks to an ethnobotanical study in the harvest region, O. elongatum used for treatment of arterial hypertension. Flavonoids are known to possess cardioprotective properties and their isolation from different medicinal plants has exhibited antihypertensive action. Wang et al. (2004) suggested that flavonoids may act in vascular system as antiarrhythmic and vasodilator agents **[8].** With accordance with this, our work aimed to evaluate the total phenolic content, and compare the vasodilatory activity of various solvent extracts from the three plants selected from Al-Hoceima province in the north of Morocco.

2. MATERIALS AND METHODS

2.1. Plant material

The plants were harvested in February 2014 in the region of Beni Ammart, a village in the province of Al Hoceima in the Rif mountains. The plants were identified by Mr. A. ENNABILI, Professor at the National Institute of medicinal and aromatic plants in taounate. The harvested plants are sorted and dried in an oven at a temperature of 40 ° C for 24 hours, then the leaves were separated from the rest of the sample, and then ground with a grinder to obtain a fine powder plant. These powders are stored in glass vials.

2.2. Ultrasound-assisted extraction (UAE)

Extraction was carried out in an ultrasonic device (CY-500 sonicator, JP Selecta S.A. Spain) at 500 W and at a frequency of 20 Hz**.[9]** The dried powder sample was extracted in a 250 mL beaker at the ambient temperature. Ultrasonic probe was directly inserted into the beaker about 4 cm under the surface of the mixture to provide direct contact with the sample for 60 min. At the end of sonication, the suspension was cooled to ambient temperature and then filtered through filter paper (Whatman no. 4) to remove solid debris. The solvent was removed by a rotary evaporator at temperature lower than 40°C. The prepared extract was kept in the dark at 4°C until further.

2.3. Dosage of total polyphenols

Total polyphenols content was estimated using (FC) assay which is widely used in routine analysis.**[10]** Briefly, all samples and gallic acid were dissolved in 50% (v/v) aqueous methanol. Samples (0.5 mL) were placed into test tubes and then 2.5 mL Folin-Ciocalteu reagent (10%, v/v, in water) solution and 7.5 mL sodium carbonate (20%, v/v, in water) solution were. The tube contents were mixed and allowed to stand for 90 min at ambient temperature. The absorbance was measured at 750 nm and the total phenolic content was expressed as gallic acid equivalents (GAE) in mg per g of extract.

2.4. Dosage of total flavonoids

The total flavonoid content was determined using the method as adapted by Arvouet-Grand and al. **[11]** 1.0 ml of 2% aluminum trichloride ($AlCl_3$,$6H_2O$) in methanol was mixed with the same volume of the extract solution. Absorption readings at 430 nm using Perkin Elmer UV-VIS spectrophotometer were taken after 10 min against a blank sample consisting of extract solution with 1.0 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with Quercetin and expressed as mg of Quercetin equivalents per gram of sample.

2.5. Vasodilatory activity

2.5.1. Work on the mesenteric vascular bed (MVB) Wistar rats

The isolated mesenteric bed turned out to be a useful vascular tissue for the studies of vasoreactivity since it contains a representative population of arteries and arterioles and seems like a good resistance area very involved in the regulation of blood pressure. In our study, we chose to explore the vasodilator effect of the three plants Origanum elongatum, Cistus salviifolius and Cistus laurifolius at the MVB. In this work, vasoreactivity of MVB incubated in the presence of the three plants with a synthesis inhibitor endothelial vasodilators factors, was studied in response to a vasoconstrictor α-mimetic: phenylephrine (PHE). Once the vasodilator effect of the three plants is established on preparations precontracted to the PHE, we examined the possible involvement of MVB and vasodilator factor (acetylcholine Ach) in this regard.

2.5.2. preparation of mesenteric vascular bed

The rats were anesthetized with urethane (50 mg/Kg), then the abdominal cavity is opened, the superior mesenteric artery is identified, a hemi section is carried out at its origin with the abdominal aorta through which is introduced a hypodermic needle **[12].** The infusion is immediately started to avoid anoxia mesenteric bed with a peristaltic pump (Pharmacia Bio Tech) at a constant flow (2 ml/min) **[13,14].** The infusion solution, Krebs Heinseleit consist (mM) of: NaCl 118.3; KCl.4.7; $CaCl_2$.$2H_2O$ 2.5; $MgSO_4$. $6H_2O$ 1.2; $NaHCO_3$ 25.0; $KH_2PO_4$1.2; Glucose 11.1 and the pH is adjusted to 7.4. To maintain this pH and oxygenate the perfusion solution, the solution is bubbled with carbogen (95% O_2 and 5% CO_2). The temperature was maintained at 37 ° C. Once the infusion started, the BVM is minutely isolated with fine scissors on the edges of the intestines and placed on a petri dish. Once the BVM isolated, the rats were sacrificed by cervical dislocation. The superior mesenteric artery emits a large number of branches, which are also divided into several blood vessels to provide irrigation mesenteries **[15].** Vascular responses were detected as changes in perfusion pressure **[16],** which was measured continuously with a pressure sensor (Capto 844) and recorded on an oscillograph (Harvard Apparatus Limited). The perfusion variations on mmHg are measured using a pressure transducer (Model TCB 100, Millar, Houston, TX). After 30 minutes of stabilization, the BVM receives injections of phenylephrine (50 nmol) every 5 minutes, an α_1 receptor agonist, until the answers are reproducible, then relaxed with 30 µl of acetylcholine to confirm the integrity of BVM. The increasing concentrations of the extracts of the three plants were added cumulatively in the environment of survival.

3. RESULTS AND DISCUSSION

3.1. Total polyphenols and flavonoids

The result of total polyphenols and flavonoids contents of the four crude extracts is given in (Table 1). The determination of the polyphenols was carried out as a function of a linear calibration curve (y = ax + b) of a gallic acid solution at different concentrations (0 to 100 g / 100 ml). The flavonoid content of each extract is expressed in milligrams equivalent to Quercetin per gram of extract (mg EQ/g of extract) **[17].**

Table 1: Polyphenols and Flavonoids Quantities in each extract of plants.

Plant material	Extracts	Polyphenols µg (EAG)/100mg)	Flavonoïdes µg (EQ)/mg)
Plante1 : Origanum elongatum	-Ethyl acetate	(130,00 ± 3,05)	(5,00±0,89)
	-Methanol	(153,22 ± 2,67)	(8,16±0,71)
-Plante2 : Cistus salviifolius	- Ethyl acetate	(80,00 ± 1,05)	(4,83± 0,72)
	-Methanol	(98,84 ± 0,99)	(5,39±0,68)
-Plant3 : Cistus laurifolius	- Ethyl acetate	(46,63 ± 0,38)	(5,02 ± 0,26)
	-Methanol	(64,54 ± 0,33)	(5,54± 0,90)

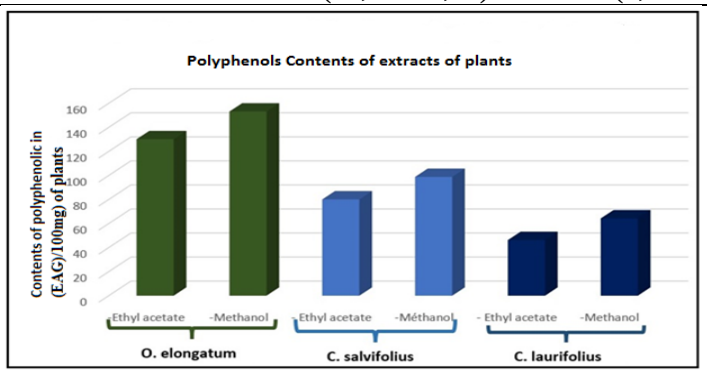


Figure 1: Polyphenols Contents of extracts of plants.

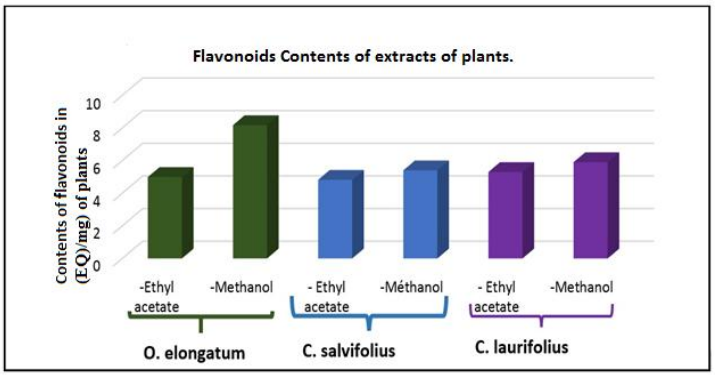


Figure 2: Flavonoids Contents of extracts of plants.

Total phenolic contents (TPC) and flavonoids contents (TFC) in plant extracts increase with solvent polarity. Indeed, methanolic extracts were found richer in TPC and TFC than ethyl acetate extracts especially Origanum elongatum which presented (153,22 ± 2,67) in methanolic extract against (130 ± 3,05) mg GAE / g dry in ethyl acetate one's. This observation has been proved for others species of Origanum Majorana by Kamble et al **[18].** The TPC of extracts was in the order of Origanum elongatum > Cistus salviifolius > Cistus laurifolius. A higher polarity solvent like methanol permits to extract more phenolic compounds **[19].** A similar tendency was observed for the TFC, the lowest amount was recorded in ethyl acetate extracts (4,83±0,72) to (5±0,89) mgEQ/ g dry weight against methanolic extract (5,02 ± 0.26) to (8,16±0,71) mgEQ/ g dry weight .The highest TFC were found in the leaves of Origanum elongatum, followed by Cistus salviifolius and Cistus laurifolius . In this context, other authors exhibited that the TPC and TFC are predominated with variable quantity in the same species, Rebaya and al proved that the TPC ranging from (11.96 ± 0.14) to (56.03 ± 0.06) g GAE. 100 g^{-1} in leaves C. salviifolius**.[20]** Our results were consistent with these values.

3.2. vasodilator activity

The vasodilating activity of each sample was determined by measuring the perfusion pressure (PP) of the mesenteric bed of the rat **[21].** It is determined by calculating the difference between the blood pressure before injection and blood pressure after injection of the test product. This difference is proportional to the vasodilatory activity (more the PP is higher, more the product is active). The results of the three plants studied are presented in the following lines:

3.2.1. Origanum elongatum

Note that the methanol extract O. elongatum has a vasodilating activity (PP = 50 mmHg) close to that of acetylcholine (PP = 40 mmHg) and it also induces an extension of the vasodilator response (Figure 3). The extracted in ethyl acetate O. elongatum (PP = 20 mmHg) is less active than the reference (PP = 40 mmHg). We also note a continuation of the vasodilator response after injection of this extract (Figure 4). For O. elongatum, the methanol extracts (PP = 50 mmHg) were more active than those in the ethyl acetate (PP = 20 mmHg). The O. elongatum belongs to the Elongatispica, **[22]** which is characterized by species whose EO contain carvacrol, , γ-terpinene and para-cymene.**[23, 24**] Our results show that O. elongatum from Moroccan Rif has a composition comparable to other Origanum species except for Thymol which is present in very small quantities. The high content of thymol and its isomer carvacrol is correlated with several biological activities **[25, 26].**

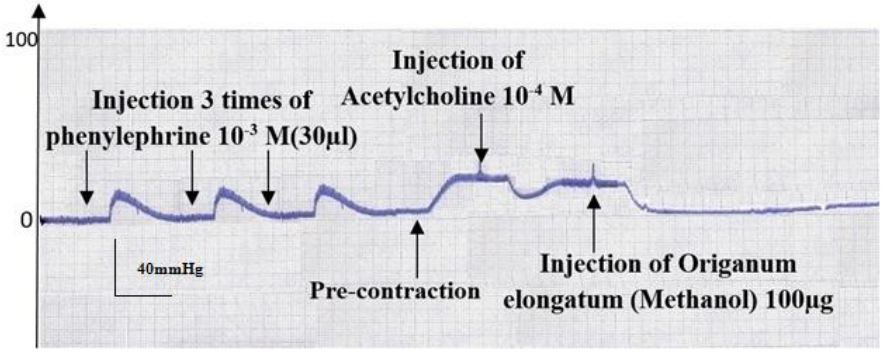


Figure 3: Representative perfusion pressure (mmHg) trace showing the vasodilator effect of O. elongatum methanol extract on MVB

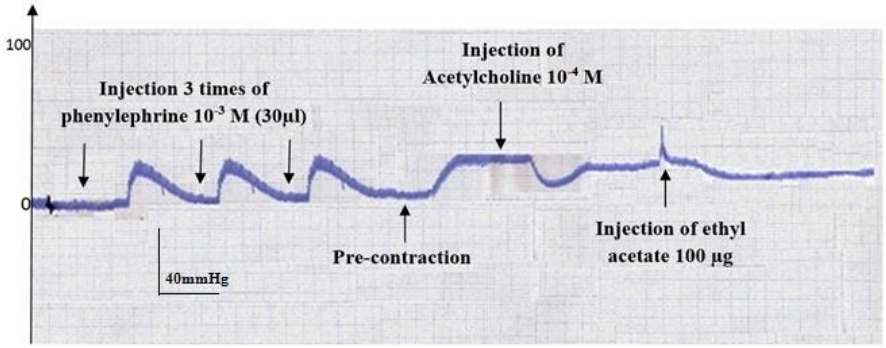


Figure 4: Representative perfusion pressure (mmHg) trace showing the vasodilator effect of O. elongatum methanol extract on MVB

3.2.2. Cistus salviifolius

The injection of the methanolic extract of C. salviifolius of the doses tested (30 to 300 µg/ml) induced a vasodilating activity proportional to the dose injected.

The hypotensive effect was observed from 100 µg /ml, which lowers the pressure by 25 mmHg. At 300 µg/ml a response equal to the activity of acetylcholine (PP=40mmHg) was obtained.

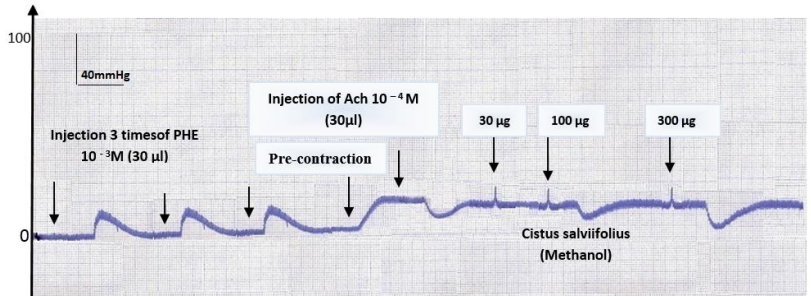
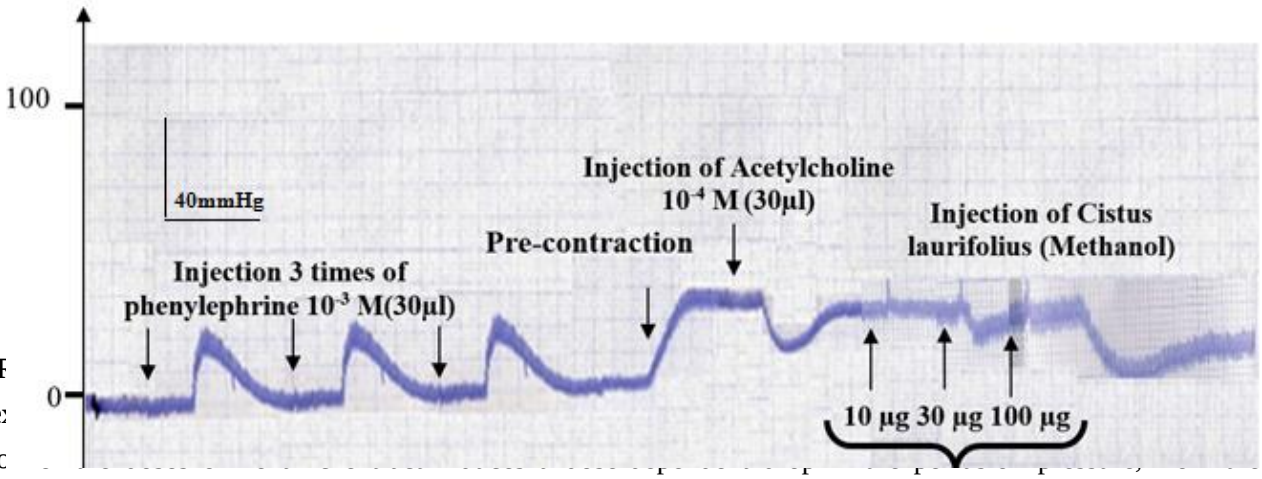


Figure 5: Representative perfusion pressure (mmHg) trace showing the vasodilator effect of C. salviifolius methanol extract on MVB

3.2.3. Cistus laurifolius

The injection of increasing doses of methanolic extract of Cistus laurifolius induced a dose-dependent drop on the infusion pressure. From 30 µg/ml, a pressure decrease of 20 mmHg is observed, and at 100 µg/ml a decrease of 40 mmHg is observed. (Figure 5). The vasodilatory activity of methanolic extract of Cistus laurifolius at 100 µg/ml is the same as that of 10^{-4} M acetylcholine (40 mmHg).



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concentration 30 µg/ml (drop of 20 mmHg). At 100 µg/ml the pressure decreases by 40 mmHg (same PP as acetylcholine), at 300 µg/ml It decreases by 80 mmHg and finally at 1 mg/ml a decrease of 120 mmHg is observed (Figure 6).The hexanic extract of Cistus laurifolius achieves the same hypotensive effect as the reference at concentration of 100 µg/ml, then this effect increases with increasing concentration.

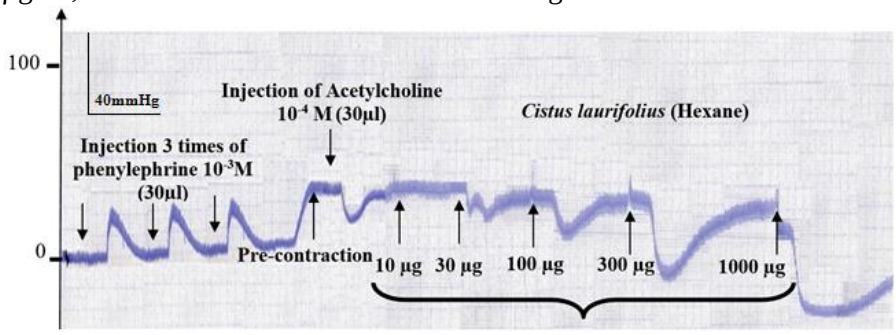

Figure 7: Representative perfusion pressure (mmHg) trace showing the vasodilator effect of C. laurifolius Hexane extract on MVB

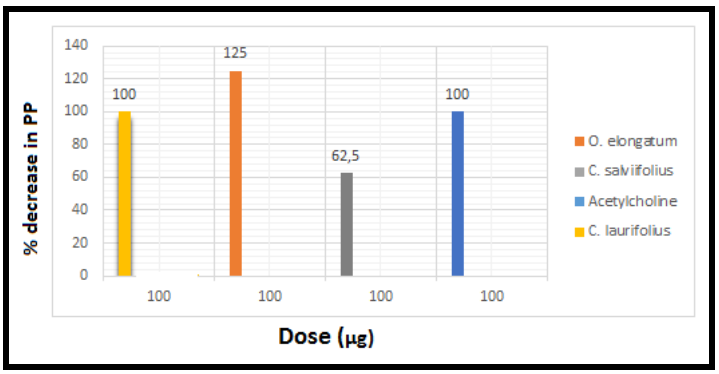

Figure 8: Vasodilator effect of O. elongatum, C. salviifolius, C. laurifolius methanol extracts on MVB

The vasodilator effect of methanolic extract of O. elongatum was more effective than that of C. laurifolius itself was more effective than that of C. salviifolius extract verified in the present study. However, the O. elongatum's extracts effects on MVB reached 125% potency at 100 µg, the effects of C. laurifolius extracts on MVB reached 40% potency at 100 µg, the effects of C. salviifolius extracts on MVB reached 65,5% potency at 100 µg (Figure 8). The similarities respecting to response and vasodilator effect of these species could be related to presence of common secondary metabolites. However, research by Ferreira et al. [27], showed that the flavonoids of methanolic extracts of contributed to endothelium-dependent vasodilator activity in the mesenteric environment of rats. Endothelium independent Vasodilator action has also been verified in quercetin flavonoid studies. Finally, by comparing the vasodilatory activity of three plants, we find the same results of antioxidant activity [28]. In summary, among the three plants tested, only methanolic extract of Origanum elongatum, cistus laurifolius and Cistus salvifolius showed vasodilatory activity. Therefore, we can conclude that the vasodilator activity found among the three plants studied was due to their antioxidant power.

CONCLUSION

Although the methanolic and hexanic extracts of Origanum elongatum, cistus laurifolius and Cistus salvifolius showed a vasodilating action. The phytochemical analysis of the different extracts obtained by sonication revealed the presence of polyphenols and flavonoids. We also have demonstrated a correlation between the content in polyphenols and in flavonoids in our extracts, which probably are the responsible agents for the vasodilator effect.

However, phenolic compounds and flavonoids were detected for the plants it as a possible natural source for obtaining bioactive compounds useful against hypertension.

REFERENCE

[1] C. Nejjar, M. Arharbi, MT. Chentir, R. Boujnah, Journal of hypertenssion 31 (1), 49-62, 2013.

[2] Burns J, Gardner PT, O'Neil J,Crawford S, Morecroft I, McPhail DB, Lister C, Matthews D, MacLean MR, Lean ME, Duthie GG, Crozier A (2000),Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines., J Agric Food Chem. 2000 Feb;48(2):220-

[3] T L Yue, H Y Cheng, P G Lysko, P J McKenna, R Feuerstein, J L Gu,K A Lysko,L L Davisand G Feuerstein (1992),Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger the journal of pharmacology :192-98.

[4] G. Z. Feuerstein and R. R. Ruffolo JR (1995), Carvedilol, a novel multiple action antihypertensive agent with antioxidant activity and the potential for myocardial and vascular protection European Heart Journal :38-42
[5] Sidibé F. (2003). Etude phytochimique et pharmacologique de Stereospermum kunthianum Cham. (Bignoniaceae). Thèse de Pharmacie, Bamako ; 79 P.
[6] Huang, D., Ou, B., Prior, R. L. (2005) The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry. 53, 1841-1856
[7] Oualili Hanane, Rchid Halima et Nmila Rachid.Phytochemical Screening and Antioxidant Activity
of: Origanum elongatum and Cupressus atlantica Two Endemic Plants of Morocco Int. J. Pharmacognosy and Phytochem. Res., 2018; 10(5):196-200
[8] WANG, S.; DUSTING, G. J.; WOODMAN, O. L.; MAY, C. N. Selective vasodilator and chronotropic actions of 3`, 4`-dihydroxyflavonol in conscious sheep. Eur. J. Pharmacol., v.491, n.1, p.43-51, 2004.
[9] T L Yue, H Y Cheng, P G Lysko, P J McKenna, R Feuerstein, J L Gu,K A Lysko,L L Davisand G Feuerstein (1992),Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger the journal of pharmacology :192-98.
[10] G. Z. Feuerstein and R. R. Ruffolo JR (1995), Carvedilol, a novel multiple action antihypertensive agent with antioxidant activity and the potential for myocardial and vascular protection European Heart Journal :38-42
[11] Julien Ferrari (2002), Contribution à la connaissance du métabolisme secondaire des Thymelaeaceae et investigation phytochimique de l'une d'elles:Gnidia involucrata Steud. Faculté des Sciences de l'Université de Lausanne.p13.
[12] Prempeh A, Mensah-Adipoe J: Crude aqueous extract of the root bark of Zanthoxylum Xanthoxyloides inhibits white blood cells migration in acute inflammation, Ghana Med J, sept. 2008, 42(3); 117-9.
[13] Namiranian K, et al. Eur J Pharmacol. 2001, Mesenteric vascular bed responsiveness in bile duct-ligated rats: roles of opioid and nitric oxide systems.
[14] Rachid Ben Cheikh, Jean-Claude Feray, Abdelillah Alaoui, and Bertrand Crozatier, Thromboxane A2 in Vasomotor Effects of Phenylephrine,Acetylcholine, and Bradykinin in Rat Mesenteric Bed. J Cardiovasc Pharmacol™, 2002, Vol. 40, No. 2:255–264.
[15] Chokri A, El Abida K, Zegzouti YF, Ben Cheikh R. Endothelium-dependent vascular relaxation induced by Globularia alypum extract is mediated by EDHF in perfused rat mesenteric arterial bed. Can J Physiol Pharmacol. 2012 May;90(5):607-16
[16] Garas, S. et Zafari, A.M. (2009). Myocardial Infarction. EMedicine
[17] Arnous, A., Makris, D. P., & Kefalas, P. (2002). Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. Journal of Food Composition and Analysis, 15, 655–665
[18] Kamble V. and Ganga Rao B. Effect of Roots of Origanum majorana in Methanolic and Ethyl Acetate Extracts on CCl4-Induced Hepatotoxicity in Rats. Int. J. Pharm. Sci. Drug Res., 2018; 10(2): 51-56
[19]. Falleh H., Ksouri R., Lucchessi M. E., Abdely C. and Magné C. Ultrasound-Assisted Extraction: Effect of Extraction Time and Solvent Power on the Levels of Polyphenols and Antioxidant Activity of Mesembryanthemum edule L. Aizoaceae Shoots. Trop. J. Pharm. Res., 2012; 11(2): 243-249.
[20] Rebaya A. Belghith S. I., Cherif J. K. and TrabelsiAyadi M. Total Phenolic Compounds and Antioxidant Potential of Rokrose (Cistus salviifolius) Leaves and Flowers Grown in Tunisia. Int. J. Pharmacognosy and Phytochem. Res., 2016; 8(2): 327-331.

[21] Amoussou G. Ayéwodé, Rachid Bencheikh.Developpement de nouveaux vasodilatateurs à partir d'une plante togolaise Fagara Zanthoxiloides. Laboratoire de Molécules Bioactives, Faculté des Sciences et Techniques (FST) de Fès, Université Sidi-Mohammed-Ben-Abdellah
[22] Emiliano, A. F. Efeito vasodilatador do extrato hidroalcoólico da Alpinia zerumbet (Pers.) Burtt et Smith no leito vascular mesentérico de rato. Rio de Janeiro, 2002. 74 f. [Dissertação
de Mestrado. Faculdade de Ciências Médicas. Universidade do Estado do Rio de Janeiro].
[23] Figuerdo G, Cabassu P, Chalchat JC, Pasquier B. Studies of mediterranean oregano populations VIII: Chemical composition of essential oils of oregano of various origins. J Essent Oil Res 2006;21:134-5
[24] Aboukhalid K, Lamiri A, Agacka-Mołdoch M, Doroszewska T, Douaik A, Bakha M, et al. Chemical polymorphism of Origanum compactum grown in all natural habitats in Morocco. Chem Biodivers 2016;13:1126-39
[25] Du E, Gan L, Li Z, Wang W, Liu D, Guo Y.In vitro antibacterial activity of thymol and carvacrol and their effects on broiler chickens challenged with Clostridium perfringens. J Anim Sci Biotechnol 2015;6:58
[26] Koparal AT, Zeytinoglu M. Effects of carvacrol on a human non-small cell lung cancer (NSCLC) cell line, A549. Cytotechnology 2003;43:149-54.
[27] FERREIRA, H. C.; SERRA, C. P.; ENDRINGER, D. C.; LEMOS, V. S.; BRAGA, F. C.; CORTES, S. F. Endotelium dependent vasodilation induced by Hancornia speciosa in rat superior mesenteric artery. Phytomedicine, v.14, n.7, p.473-478, 2007.
[28] K. el yousfi, H. misbahi, H. greche and M. Boudkhili. Evaluation of Phytochemical and Antioxydant Activities of Origanum, Cistus and Thymus Species. European journal of biomedical and pharmaceutical sciences, v.5, I.12, 10-14, 2018.