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BIOLOGICAL INVESTIGATIONS OF ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF *THYMUS SATUREIOIDES* COLLECTED IN TAFILALET REGION, SOUTH-EAST OF MOROCCO

Mhamed Ramchoun [1,3*], Khalid Sellam [2], Hicham Harnafi [1], Chakib Alem [3], Mohamed Benlyas [3], Lhoussaine EL Rhaffari [2], Souliman Amrani [1]

[1]Laboratory of Biochemistry, Department of Biology, Faculty of Sciences, 60000 Oujda, Morocco
[2]Laboratory of Microbiology, Department of Biology, Faculty of Sciences & Techniques 52000 Errachidia, Morocco
[3]Laboratory of Biochemistry, Department of Biology, Faculty of Sciences & Techniques 52000 Errachidia, Morocco

***Corresponding author e-mail:** ramchoun_10@yahoo.fr

ABSTRACT

The present study was carried out to investigate the antioxidant an antimicrobial effect of different extracts of *Thymus satureoïdes*. The FRAP assay shows that the antioxidant activity was between 07.03±0.29 and 117.51±6.46 mmol Trolox/g of extract. The IC_{50} values from different extracts of *Thymus satureoides* were between 0.41±0.005 and 43.89±2.46 mg/mL of extract. The different extracts were also, demonstrated the important significantly ($p<0.001$) reduction of the malondialdehyde. The total polyphenols extract was particularly found to possess stronger antimicrobial activity.

Keywords: *Thymus satureoides*, polyphenols, antioxidant, antimicrobial, malondialdehyde.

INTRODUCTION

Plants are good sources of active natural products that differ widely in terms of structure and biological properties so; they can be used for various applications, especially as food additives and health promoting ingredients. For the reason, during last few decades, they have been become a subject for study of bioactive compounds[1-2]. Our groups has worked on four varieties of thyme and showed that gross these plants possess a very important antioxidant capacity[3-4], in particular *Thymus satureoides*. A growing amount of evidence has shown that the oxidative stress and free radicals play the important roles in the etiology of some chronic diseases. Epidemiological studies have revealed that the consumption of antioxidants is positively associated with the reduced risk of developing chronic and ageing related diseases. On the other hand, synthetic antioxidants have been shown to be potentially toxic. Therefore, there is a growing interest in searching for antioxidants naturally present in plants[5]. Numerous crude extracts and pure natural compounds have been reported to have antioxidant and radical scavenging activities[6]. Phytochemicals in fruits and vegetables act synergistically and additively to provide potential health benefits against chronic diseases by inhibiting the harmful effects of free radicals[7].

Over the recent years, there has been growing interest in naturally occurring phytochemical compounds with anticancer potential. These products are relatively not toxic, inexpensive and available in an ingestive form. Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity[8-9]. Herbs are used in many domains, including medicine,

nutrition, flavouring, beverages, dyeing, repellents, fragrances, cosmetics[10]. Many species have been recognized to have medicinal properties and beneficial impact on health, e.g.antioxidant activity, digestive stimulation action, antiinflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anticarcinogenic potential[11]. Also, its can prevent oxidative damage via a number of different mechanisms, such as free radical scavenging, transition metal chelation and interactions with lipid membranes, proteins and nucleic acids[12].

The thyme is an aromatic plant of the Mediterranean flora, commonly used as spices and as traditional medicine remedies. They are reported to possess some biological effects such as anti-oxydant[13-15], antibacterial[16], antifungal[17-18], antitabagism[19], giardicidal[20], antispasmodic[15] and antiaflatoxigenic properties[21]. In Morocco, the thyme is represented by many species of which certain are endemic. The flowered stem contains essentially flavonoïds (derived of apigenol and luteolol), acids phenols, in particular, cafeic and rosmarinic[22-23], tannins, resin and especially essential oil rich in chemical compounds which are responsible for the majority of its pharmacological effects[22].

The thyme has been used, in Moroccan traditional medicine, in the treatment of diarrhoea, fever, cough, infected areas and wounds. It was also used as a tonic and stimulant[24] and generally, for its anti-inflammatory properties after topical or oral administration[25]. This work was designed to evaluate the antioxidant and antimicrobial activities of aqueous extract, total polyphenols and total flavonoids extracted from *Thymus satureoides.*

MATERIAL AND METHODS

Plant material: *Thymus satureioides* was collected in April-Mai 2009 in the Tafilalet region, south-east of Morocco. The plants were determined by Dr. Ibn Tatou and a voucher specimen was deposited at the herbarium of the Scientific Institute, University Mohammed V, Rabat, Morocco (N°: RAB 77497).

Preparation of the aqueous extract: The aqueous extracts were prepared using a manner similar to that used by patients with some modifications. The dried powder from aerial parts (50 g) of the plant was exhausted in a Soxhlet extractor for 4h, filtered, and the solution obtained concentrated in a rotatory evaporator under vacuum at 60 °C. The yield of extract in terms of starting dried plant material was of 14% (w/w). The resulting crude extract was suspended in distilled water and the aliquots were stored at - 20 °C before use.

Polyphenols extraction: The polyphenols extraction was assayed by the method described by Maria et al.[8], with some modifications. The dried powder from aerial parts (50 g) of the plant was defatted with n-hexane (C_6H_{14}) in a Soxhlet apparatus. Afterwards, the residue was air-dried and extracted with methanol 80% (16 h at 45 °C). The resulting residue was resuspended in distilled water and extracted with n-butanol. The aqueous fraction (polyphenolic fraction) was concentrated and resuspended in distilled water for a battery of biologic tests.

Flavonoids extraction: The flavonoids extraction has been made by the method of Lee et al.[26], with some modifications. The dried aerial parts of the herbs (50 g) were extracted by Soxhlet in distilled water and ethanol (1:1) during 4 h at 60 °C, filtered and the aqueous phase obtained was extracted by the n-butanol and acidified by HCl 10% (pH: 3.00). The butanolic fraction has been concentrated in rotator evaporator under vacuum at 40 °C. The obtained product has been extracted three times by the mixture of distilled water /ethyl acetate (1:1) during one hour and the organic fraction has been basified by $NaHCO_3$ (pH: 9.00). After 15 minutes, the organic fraction (total flavonoids) has been evaporated driely at 40 °C, weighed and the residue was suspended in ethanol for the biologic tests.

Determination of total phenol contents in crud aqueous extract: Total polyphenols content in aqueous extracts were assayed using the Folin-Ciocalteu reagent, following Singleton and Rosis[27] method based on the reduction of a phosphowolframate-phosphomolybdate complex by phenolics to blue reaction products and slightly modified by Dewanto and al.[28], with some modifications. An aliquot of diluted sample extract was added to 3.5 ml of distilled water and 0.25 ml of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before addition of 0.5 ml of 7% Na_2CO_3. The solution was then adjusted with distilled water to a final volume of 5 ml and mixed thoroughly. After incubation in dark for 30 min, the absorbance at 725 nm was read versus the prepared blank. Total polyphenols content of the aqueous extract was expressed as milligrams of caffeic acid equivalents per gram of dry plant through the calibration curve with caffeic acid. All samples were analyzed in three replications.

Determination of flavonoids contents in crud aqueous extract: The flavonoids content in extract

was determined spectrophotometrically according to Jay and al.[29], using a method based on the formation of a complex flavonoids-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 *Thymus satureioides* extracts

Radical-scavenging activity (RSA) assay: 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[30]. The capacity to scavenge the ''stable'' free radical 2,2- diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method of Hatano and al.[31]. Various concentrations of aqueous extracts, polyphenols and flavonoids extract from *Thymus satureioides* (100 µl) were mixed with 1.9 ml of methanolic solution containing DPPH radicals (0.06 mg/ml). The mixture was shaken vigorously and left to stand for 30 min at room temperature. The reduction of the DPPH-radical was measured by monitoring continuously the decrease of absorption at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation:

$\%RSA = [(A_{DPPH} - A_S)/ A_{DPPH}] \times 100$

Where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of RSA percentage against extract concentration. Trolox were used as reference compound.

Ferric Reducing-Antioxidant Power (FRAP) assay: The method is based on the reduction of the Fe3+TPTZ (tripyridyl triazine) complex to the ferrous form at low pH[32]. This reduction is monitored by measuring at 37°C, the absorption change at 593 nm under in acid conditions (pH: 3.6).

Inhibition of Lipid Peroxidation: The level of lipid peroxidation products in tissues or plasma lipid was measured as thiobarbituric acid reactive substances (TBARS) according to the modified method of Park and al.[33]. Briefly, lipid-rich plasma obtained from mice injected with Triton WR-1339 at a dose of 600 mg/kg BW was used as substrate for oxidative process. In brief, in the control tube, plasma was incubated with distilled water only. In the second test, oxidation was inducted with 10µl of copper sulphate ($CuSO_4$; $5H_2O$) solution (0.33 mg/ml). In the third assay, oxidation was inducted by copper and aqueous extract, total polyphenols and flavonoids were added at 25, 50 and 100 mg/ml to evaluate their possible anti-oxidant property during 24 h at 30°C. Then, to each assay was added 100 µl of 8.1 % (w/v) sulphate dodecyl sodium (SDS), the mixture was stirred and incubated 60 min at room temperature. Afterward, the reaction mixture was heated at 95°C for 60 min after the addition of 250 µl of 20% trichloroacetic acid (pH 3.5) and 250 µl of 0.8% (w/v) thiobarbituric acid (TBA). After cooling, 1 ml of n-butanol was added and vortexed. The solution was centrifuged at 4500 rpm for 15 min and the absorbance of resulting coloured layer was recorded at 532 nm. The amount of TBARS was calculated as a MDA equivalent from the calibration curve of 1,1,3,3-tetramethoxypropane standard solutions and expressed as mM MDA. All measurements were done in triplicate.

Antimicrobial study of *Thymus satureioides* extracts

Screening for antimicrobial activity: The antimicrobial activity was evaluated by paper disc diffusion and dilution methods against four selected Gram-positive and Gram-negative species: *B. cereus ATCC 25923*, *E. coli ATCC 25922*, *Pseudomonas aeruginosa ATCC27853*, *Staphylcoccus aureus ATCC25923* and against the phytopathogenic fungi *Candida albicans* and *Penicillium sp.*

Diffusion method: The qualitative antimicrobial essay was carried out by the disc diffusion method[34-35]. It was performed using culture growth at 37ºC for 18 h and adjusted to approximately 105 colony forming unit per milliliter (CFU/ml). The culture medium used for the bacteria was Mueller Hinton Agar (MHA), while Sabouraud was used for growing the fungi. Five hundred microliters of the inoculums were spread over plates containing MHA or Sabouraud and a Whatman paper disc (6 mm) impregnated with 50µm of different extracts was placed on the surface of the media. They were incubated 24 h at 37ºC for the bacteria and 48 h at 37ºC for the fungi. After incubation period, the inhibition zone obtained around the disc was measured. Two controls were also included in the test; the first was prepared using a disk impregnated with same solvent as that used to dissolve the plant extracts and the second was two standard antibiotics: Ampicillin used to control the sensitivity of the tested bacteria and Nystatine to control the tested fungi.

Dilution method: The minimum inhibitory concentration (MIC) was determined by the agar-

dilution method[34]. The extracts were dissolved in sterile water for aqueous extract and 10% aqueous dimethylsulfoxide (DMSO) for organic extract to a final concentration of 10 mg/ml. Then, serial two-fold dilutions were prepared from the stock solution to give concentrations ranging from 1 to 0.125 mg/ml.

Two milliliters of each concentration was mixed with 18 ml of sterile Mueller Hinton agar for bacteria and sterile Sabouraud for fungus to obtain the following final concentrations: 125, 250, 500 and 1000 µg/ml for tested extracts. Solvent control was prepared with DMSO (10%) and sterile water and blank control was prepared by virgin media. The plates were inoculated with the bacterial and fungal suspension (50 µl per will) was incubated at 37 °C for 24 h[36]. The MIC was defined as the lowest concentration at which no visible growth was observed. Each MIC experiment was repeated three times.

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

The yields of extraction are 14%, 6% and 7% for the aqueous extract, total polyphenol and flavonoids respectively. The extract is dissolved in a minimum amount of double distilled water for biological tests. The total polyphenol and flavonoids content of the aqueous extract are shown in Table1. The total polyphenol content were 456.73±6.94 mg eq cafeic acid/g of dry plant. The flavonoids content were 172.79±2.12 mg eq the rutin/g of dry plant.

Antioxidant activities

RSA and FRAP assay: The RSA of the different extracts was evaluated using a methanolic solution of the ''stable'' free radical, DPPH. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colourless/bleached product (i.e., 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The antioxidant activities of *Thymus satureioides* is shown in Table 2. Using the RSA method the radical scavenging activity from aqueous extract IC_{50} is 0,480±0,010 mg/ml, from total polyphenols IC_{50} is 0.418±0,005 mg/ml and from total flavonoids IC_{50} is 43.891±2.467 mg/ml. The use of FRAP assay shows that the antioxidant activity from aqueous extract is 50.79±2,02; 117.51±6,46; 7.03±0.29 mmol of trolox/g dry plant from the aqueous extract, total polyphenols and total flavonoids respectively. The results are expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm. The scavenging effects of total polyphenols extract on DPPH radicals increased with the concentration increase and were high (87–100% at 0.78 mg/ml), and comparable to the RSA values obtained for the standard Trolox (96% at 0.97 mg/ml).

Malondialdehyde (MDA) assay: The determination of thiobarbituric acid reactants (TBARs) is a widely used method for investigating overall lipid peroxidation. An assay that could be used with plasma and lipid fractions would facilitate standardization of the method. The results of this test are given in figure 1. The results reported here indicate that aqueous extract, total polyphenols and flavonoids were demonstrated the important significantly ($P<0.001$) reduction of the malondialdehyde formed by the oxidative action of $CuSO_4$. But, the phenolic substances are the major family with raised the interesting anti-oxydant effect ($P<0.001$ against the oxidized control).

Antimicrobial activity against human pathogenic bacteria: The antimicrobial effects of different extracts from *Thymus satureoides* against Escherichia coli, *B. cereus*, *Pseudomonas aeruginosa*, *Staphylcoccus aureus* and against the phytopathogenic fungi *Candida albicans* and *Penicillium sp* were studied. The disc diffusion method was used to evaluate the zone of microbial growth inhibition at various concentrations of the different extracts. The minimal Inhibitory Concentration (MIC) of the different extracts was determined. The results are showed in tables 3 and 4.
The study of antimicrobial capacity of plant phenolics is well known[37-38]. The aqueous extract, total polyphenols and total flavonoids were screened for their antimicrobial properties against *E. coli, B. cereus, P. aeruginosa, S. aureus, C. albicans and Penicillium sp.* The result of disc diffusion test showed that Gram positives bacteria were the most sensitive being inhibited by each of three the extracts (table 3). In case of aqueous extract maximum

inhibition was obtained against *E. coli*, *B. cereus* and *C. albicans* at the concentration 54 µg/ml, with a inhibition zone increased from 16.4 to 24.4 mm. whereas, in case of total polyphenols was obtained against *S. aureus* and penicillium sp. at the concentration 54 µg/ml with 22.3mm and 18.2 respectively. Total flavonoids gave clear zone of inhibition against *S. aureus* and *B. cereus* 26.3 mm and 22 mm. In general, Gram negative bacteria are more resistant to polyphenols than Gram positive bacteria, perhaps due to the different cell wall composition[39]. The result of MIC are presented in table 4, all extracts possess antimicrobial activities with MIC values ranging from 62.5 to 500 µg/ml. The results are in accordance with the findings in disc diffusion assay.

DISCUSSION

The thymus polyphenols presents a strong antioxidant activity as demonstrated by both FRAP and RSA tests. The antioxidant activity of this family 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[40-41]. 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[42-43]. It have been established a highly positive relationship between total phenols and antioxidant activity in many plant species[44]. Many experimental investigations have demonstrated that a number of secondary metabolites such as polyphenols compounds extracted from medicinal and aromatic plants possess a high antioxidant potential due to their hydroxyl groups and protect more efficiently against some free radical-related diseases[45]. The antibacterial activities of the different extracts from *Thymus satureioides* were tested against human pathogenic bacteria. Also The study of antimicrobial capacity of plant phenolics is well known[37-38]. In comparison with the positive control, this plant was effective against Gram (+) and Gram (−) bacteria, with a major activity against *B. ceureus*, Concerning antifungal test, both *T. satureioides* different extracts failed to show any activity in comparison with positive control (Nystatine) excepting the highest extract concentration (54 µg/ml), from total polyphenols and total flavonoids in the *C. albicans* and *Penicillium* spp respectively, which showed weak to moderate activity. Several studies attributed the inhibitory effect of plant extracts against bacterial pathogens to their phenolics composition[46-47]. The inhibitory effect of these phenolics could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation[48]. These results suggest that the antimicrobial capacity needed, as compared to antioxidant activity which has a good efficiency with crude extracts, total polyphenols and total flavonoids of *T. satureioides*, more concentration and even purification of phenolic compounds. Purified components may be used as natural antimicrobials in food systems, as well as to prevent the growth of food borne bacteria resulting in extension of the shelf life of processed foods. The antibacterial activity of *T. satureioides* extracts may be related to the action of antibiotic compounds or to the presence of metabolic toxins. It may be related to the high level of phenolic components. Proestos et al.[49] showed that several classes of polyphenols such as phenolic acids, flavonoids and tannins serve as plant defense mechanism against pathogenic microorganisms, insects, and herbivores. In fact, the site and the number of hydroxyl groups on the phenol components increased the toxicity against the microorganisms. Our results indicate that, compared to polyphenols and aqueous extract, the flavonoids extract from *Thymus satureioides* present a lesser anti-oxydant and antimicrobial activities. However, it presents an antibacterial activity equivalent to that exhibited by total polyphenols and aqueous extract. This plant did not post a hypolipidemic activity[4]. But, it posses the important antimicrobial activity interest than defense against the microorganisms. The results found are encouraging for further assessment to elucidate the mechanism of action and to identify the bioactive compounds implicated in the antioxidant and antimicrobial effects of this plant. In conclusion, several antioxidant assays, and antimicrobial activity methods were utilized in order to evaluate the biological proprieties. Various extracts of *T. satureioides* showed different levels of total polyphenols, total flavonoids and antioxidant activity. There was a strong linear relationship between total phenolics and antiradical capacity. High phenolic content is thus an important factor in determining the antioxidant activity of this plant. Moreover, *T. satureioides* total flavonoids exhibited an interesting power against several human pathogenic bacteria, possibly due to their specific composition. The action of the aqueous extract, total polyphenols or flavonoids are not limited to inhibit the free radicals, but it also seems to have an influence on the structural stability of the erythrocyte membrane. As a dietary species, *T. satureioides* appears interesting with respect to its antimicrobial activity, but also as a good source of health-promoting polyphenols such as flavonoids.

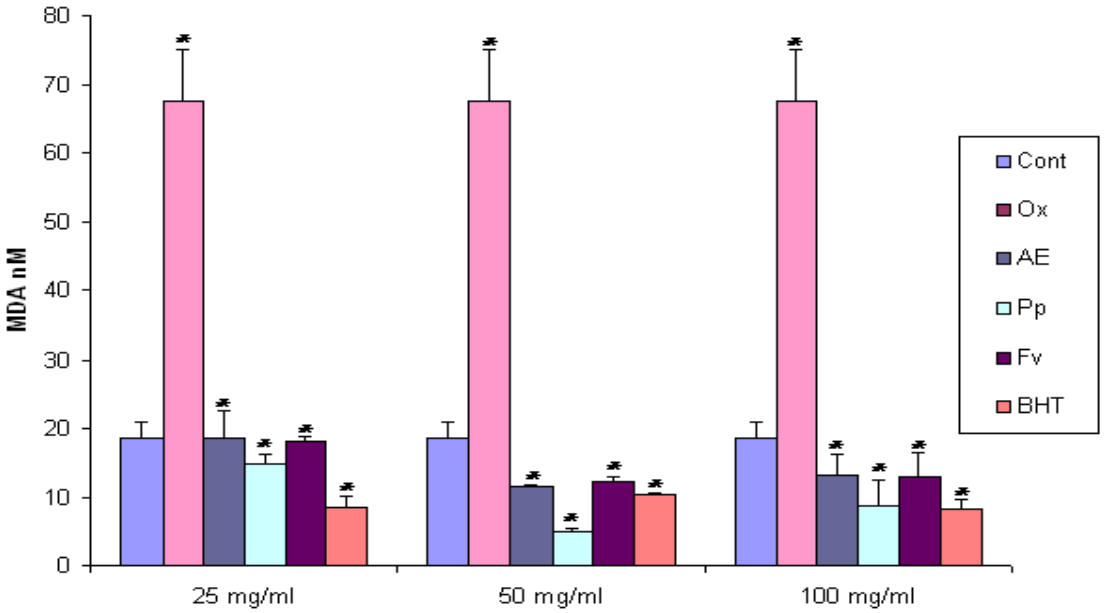

Figure 1: Malondialdehyde (MDA) assay at different concentrations of aqueous extract, total polyphenols and flavonoids from *Thymus satureioides*. Cont: control no oxidized, Ox: oxidized control, AE: aqueous extract, Pp: total polyphenol, Fv: flavonoids. BHT: Butylated Hydroxytoluene. *P <0.001 (Ox is compared with Cont. AE, Pp and Fv are compared with Ox).

Table1.Total polyphenols and flavonoids content of *Thymus satureioides* aqueous extract.

Extracts	*Total polyphenols*[a]	*Flavonoids*[b]
Aqueous extract	456.73 ±6.94	172.79 ±2.12

Values are expressed as mean ± S.E.M. from three assays.
[a] Expressed as mg caffeic acid/g dry extract.
[b] Expressed as mg rutin/g dry extract.

Table2. Antioxidant activities of aqueous extract, total polyphenols and flavonoids from *Thymus satureioides.*

Extracts	*RSA (IC50 (mg/ml E))*	*FRAP (mmol trolox/g E)*
Aqueous extract	0.48±0.01*	50.79±2.02
Total polyphenols	0.41±0.005***	117.51±6.46
Flavonoids	43.89±2.46***	7.03±0.29
Trolox	0.51± 0.01	44.33±7.55

FRAP: Ferric reducing anti-oxydant power; RSA: Reducing scavenging activity.
*RSA (***P <0.001 Total polyphénols and Flavonoïds versus Trolox.. *P<0.05 Aqueous extract versus Trolox.*

Table 3. The quantization of antimicrobial activity for extracts plant measured by the agar diffusion method.

Bacterial and fungal species	Diameter of inhibition zone (mm)													
	Thymus satureioides extracts												Antibiotics	
	EA(µg/ml)				TP(µg/ml)				TF(µg/ml)				Nys**	Amp***
	6.25	13.5	27	54	6.25	13.5	27	54	6.25	13.5	27	54		
Escherichia coli ATCC25922	na	na	14,66±0,4	16,33±1,52	na	11,58± 0,80	12,66± 1,4	13,6± 2,1	9,0± 1,66	11,5± 0,2	12,3± 0,5	13,5± 0,33	nd	20,6 ± 1,35
Pseudomonas aeruginosa ATCC27853	na	8,2±1,8	12,02±1,0	14,2± 0,66	8,0± 1,02	11,5± 1,0	12,23± 1,66	14,6± 1,8	10,1± 0,8	12,3± 0,5	14,35± 0,44	15,2± 1,2	nd	22,5± 1,54
Bacillus cereus ATCC25923	12,22± 1,25	15,33± 0,8	22,4± 0,9	24,4± 1,0	10,23± 0,8	12,4± 1,4	14,0± 1,3	16,3± 1,5	14,5± 1,2	18,5± 0,8	20,4± 0,8	22,0± 1,3	nd	21,9± 1,8
Staphylcoccus aureus ATCC25923	9.4± 0.8	10,4± 1,5	10,8± 1,44	14,22± 2,1	12,8± 1,2	16,5± 1,0	18,8± 2,0	22,3± 0,5	16,2± 2,1	19,7± 0,66	22,3± 1,6	26,3± 2,01	nd	26,8± 2,1
Candida albicans	8,55± 0,5	11,66± 1,6	15,22± 0,6	16,6± 0,8	na	na	10,5± 0,54	14,2± 0,66	10,0± 1,8	12,5± 0,66	13,2± 1,2	14,2± 0,33	22,65± 1,2	nd
Pencillium sp.	na	8,23± 2,1	11,66± 0,5	14,33± 0,66	11,2± 0,5	13,8± 0,88	15,9± 1,7	18,2± 0,8	12,2± 0,7 b	15,6± 1,55	16,4± 0,39	17,4± 0,38	18,55± 0,66	nd

AE: Aqueous extract. TP: Total polyphenols. TF: Total Flavonoids
**Nys: Nystatin (30µg/disc) ***Amp: Ampicillin (10µg/disc)
(na): No activity detected. nd : not determined.
ATCC: American Type Culture Collection.

Table 4. The minimal inhibitory concentration (MIC) of extracts plants on pathogenic microorganisms. Antibiotics (ampicillin and Nystatin) were used as the positive controls.

Bacterial and fungal species	Minimum inhibitory concentration (µg/ml)				
	Thymus satureoides extracts			Antibiotics	
	EA	TP	TF	Nys	Amp
Escherichia coli ATCC25922	500	> 500	> 500	nd	25
Pseudomonas aeruginosa ATCC27853	> 500	500	250	nd	15
Bacillus cerus ATCC25923	250	250	125	nd	0,5
Staphylcoccus aureus ATCC25923	125	125	62,5	nd	0,5
Candida albicans	250	250	250	5	nd
Pencillium sp.	500	250	125	10	nd

AE: Aqueous extract. TP: Total polyphenols. TF: Total Flavonoids
Nys: Nystatin, Amp: Ampicillin. nd : not determined
ATCC: American Type Culture Collection.

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