

ANTIBACTERIAL ACTIVITY OF THE AQUEOUS, METHANOL, ACÉTATE D'ÉTHYLE, ETHANOL AND CHLOROFORM LEAF EXTRACTS OF *THYMUS WILLDENOWII*

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ABSTRACT

At present, the raw extracts of plants begin to have a lot of interest as potential source of bioactive natural molecules. The objective of this study for their possible use as alternative for the management of infectious diseases. The aim of the study is to assess the antibacterial activity of extracts (chloroform (Chl), ethyl acetate (acet), ethanol (etoh), methanol (Met) and aqueous (aq) of *Thymus willdenowii*. The antibacterial activity was determined by the method of distribution in well towards ten bacterial strains (gram-positives and gram-négatives). The results obtained show that both extracts (EtOH) and (Chl) *Thymus willdenowii* possess an antibacterial activity on all the strains tested, but at high concentrations. By cons, other extracts (EtAc), (Met) and (Aq) were found inactive on all bacterial strains tested.

KEYWORDS: Crude extracts, Medicinal plants, Antibacterial activity, *Thymus willdenowii*.**INTRODUCTION**

Since ancient times, man recognizes the use of plants. Almost all the products used by men to relieve their ailments found their origin in the reign végétal. Almost all the products used by men to relieve their ailments found their origin in the plant kingdom. Indeed, medicinal plants represent an important therapeutic source worldwide. The therapeutic property is due to the presence of hundreds or even thousands of naturels bioactifs compounds called secondary metabolites. These molecules are known to play a major role in the adaptation of plants to their environment, but also an important source of active pharmaceuticals.

The kind of thyme is widely used in traditional medicine, it has antibacterial properties (Essawi, 2000. Dob et al, 2006), antifungal (Soliman et al., 2002), antitabagism (Carlini et al., 2006), giardicidal (Amaral et al., 2006) and antioxidant activities (Tepe et al., 2005).

Thymus willdenowii is a plant met the High Atlas, Middle Atlas, Anti-Atlas Saharan Atlas, Plain and platters of Morocco Oriental and Rif. This people thyme forests, scrub and matorrals low mountains and plateaus.

The decoction of *Thymus willdenowii* is used to treat gastrointestinal infections, liver disease, colitis, nephritis, coughs, bronchitis, infections of the gorged and mouth, flu, colds, coryza, colds. It is also a tonic and general

antiseptic (Bellakhdar 1997). El Rhaffari, (2002) showed that the decoction of the flowering plant is consumed to treat intestinal worms, herbal tea flowering tops is used to treat fever, diarrhea, fatigue, rheumatoid arthritis, cooling, microbial infection, spasms and colic, fermentation, bronchitis, asthma, colds, gall insufficiency. The *Thymus willdenowii* has many properties: Activity antiseptic, antimicrobial, antioxidant, antispasmodic (El Rhaffari, 2008) and anti-inflammatory (Ismaili, 2001). This work aims to study the antibacterial activity of various organic extracts *Thymus willdenowii*.

MATERIAL AND METHODS**Plant Material**

Thymus willdenowii was collected during May-June 2014 in the Khénifra region. This plant was authenticated by Mrs. Leila NASIRI, Professor at the Faculty of Sciences of Meknes. The plant material was dried in the shade and protected from moisture at ambient temperature for a few days before being crushed and subsequently stored until use.

Different extracts

The chloroform extracts, ethyl acetate, ethanol, methanol and aqueous different plants are obtained by a chemical fractionation by successive treatment with different solvents of increasing polarity (chloroform, ethyl acetate, ethanol, methanol and water).

The extracts obtained are recovered after complete evaporation of the solvent by means of a rotary evaporator. The residues obtained are stored at 4 °C. before carrying out the antibacterial tests.

Microorganisms tested

The antibacterial activity was evaluated against Gram-positive bacteria (*Staphylococcus aureus*) and Gram negative (*Escherichia coli* ATCC 25921, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Salmonella enteritidis*, *Pseudomonas putida*, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa*, *Proteus mirabilis*). All bacterial strains were provided by the hospital's microbiology laboratory Mohamed V Meknes. These bacteria are maintained by subculture on favorable nutrient agar for growth for 24 hours in the dark at 37 °C.

Antibacterial Activity

Preparation of inoculum

Pure colonies of different well-isolated bacterial strains were transferred to tubes containing 2.5 ml of physiological water in order to have bacterial suspensions with a concentration of 10^7 CFU / ml.

Well Dispersion Method

The Mueller-Hinton Agar medium was poured into sterile Petri dishes (diameter 9 cm), the dishes were dried at 37 °C. for 30 minutes before use. The inoculation is carried out by swabbing on petri dishes. The swab is soaked in the suspension and the agar is subsequently seeded with 1 ml of bacterial suspension.

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It is allowed to dry for 3 to 5 minutes, then wells are placed on the bottom of the box, these wells are filled with 50 µl of extract or essential oil of the plant which it is desired to test for the different dilutions (0.01-1 g / ml) for the extracts. They are placed in a cold place at 4 °C. for 30 minutes to facilitate the diffusion of the extracts especially for the essential oil and to prevent its immediate volatility. Disks of negative controls are impregnated with DMSO. Standard disks containing the reference antibiotic (Gentamicin, 10 µg per disk) serve as positive controls. The Petri dishes are incubated at 37 °C. for 18 to 24 hours. The tests were repeated three times for each antibacterial test.

RESULTS AND DISCUSSION

The diameter of the inhibition zone differs from one bacterium to another and from one extract to another. The variation in the antimicrobial activity of the extracts explains the variations in their chemical compositions.

Table 1: Antibacterial activity of the various extracts of *Thymus willdenowii*.

	Diameter of the inhibition zone *(mm)																									GN
	Chloroform extract					Extract Ethyl acetate					Ethanol extract					Methanolic extract					Aqueous extract					
	1 g/ml	0,75 g/ml	0,5 g/ml	0,25 g/ml	0,01 g/ml	1 g/ml	0,75 g/ml	0,5 g/ml	0,25 g/ml	0,01 g/ml	1 g/ml	0,75 g/ml	0,5 g/ml	0,25 g/ml	0,01 g/ml	1 g/ml	0,75 g/ml	0,5 g/ml	0,25 g/ml	0,01 g/ml	1 g/ml	0,75 g/ml	0,5 g/ml	0,25 g/ml	0,01 g/ml	
E.c	12,33 ±0,58	10,67 ±1,15	8± 1,00			-	-	-	-	-	11,67 ±1,53	10,33 ±0,58	-	-	-	-	-	-	-	-	-	-	-	-	-	24
E.c ATCC- 25921	11± 1,00	9,33±0. 58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23
K p	23,67 ±1,15	19,33± 0,58	10,67± 0,58	9± 1,00	8,33± 1,53		13± 1,15	11,33 ±1,00	10,67 ±0,58	8 ± 0,57	18,33 ±0,58	15,67 ±0,58	14± 1,00	11,67 ±1,53	8± 1,00	12,67 ±1,15	11,33 ±0,57	10,67 ±1,00	9,33 ±1,53	8,33± 0,58	13,33 ±1,00	11,67 ±1,15	10± 0,57	9,33 ±1,53	-	22
E. a	11,33 ±0,58	9,33±1, 15	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19
S.a	15± 1,00	14,33± 0,58	-			-	-	-	-	-	12,67 ±1,53	11± 1,00	-	-	-	-	-	-	-	-	-	-	-	-	-	20
P. r	10± 0,57	9,67± 01,15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. a	11,33 ±1,53	10,67± 1,15	-			-	-	-	-	-	10± 1,00	9,67± 1,53	-	-	-	-	-	-	-	-	-	-	-	-	-	20
P. a ATCC 27853	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18
P. p	11,33 ±0,58	10,67± 1,15	7± 1,00			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. e	19,67 ±0,53	14± 0,15	12,33 ±1,15			-	-	-	-	-	16± 1,00	12,33 ±0,58	-	-	-	-	-	-	-	-	-	-	-	-	-	12

*Diameter of the zone of inhibition produced around the wells

CN: Gentamicine; S.a: *Staphylococcus aureus*; E.c.: *Escherichia coli*. ECATCC- 25921: *Escherichia coli* ATCC- 25921; P.a.; *Pseudomonas aeruginosa*; P.a.; *Pseudomonas aeruginosa* ATCC 27853 ; KP : *Klebsiellapneumoniae*, Ea; *Enterobacteraerogenes*. Se; *Salmonella enteritidis* Pp; *Pseudomonas putida* Pm; *Proteus mirabilis*. Values are the average of three replicates. (-): no inhibition.

According to the results obtained, the chloroform extract (F1) and the ethanolic extract (F3) at concentrations 1 and 0.75 g / ml *Thymus willdenowii* are the two most active extracts against practically all the bacterial strains tested. While the extracts of ethyl acetate, methanol and aqueous were found to be inactive against all the strains tested.

The chloroform extract of *Thymus willdenowii* exerce has an important antibacterial activity against Gram-positive bacteria and Gram-negative bacteria tested. The most marked inhibitions ($23, 67 \pm 1.15$ mm, 19.33 ± 0.12 mm) were obtained with concentrations 1 and 0.75 g / ml, respectively, compared to the standard antibiotic (22 mm).

The ethanolic extract of this plant also exhibits a broad inhibitory effect against *K. pneumoniae*, *S. enteritidis*, *P. aeruginosa*, *E. coli* and *S. aureus*, this effect being manifested by inhibition diameters of $18, 33 \pm 0.58$; 16 ± 1.00 ; 10 ± 1.00 ; 11.67 ± 1.53 ; 12.67 ± 1.53 mm respectively for a concentration of 1 g / ml. On the other hand, it is inactive against the rest of the bacterial strains.

Our results agree with those of the literature, indeed, thymic extracts showed a broad antibacterial activity by inhibiting the growth of both Gram-positive and Gram-negative bacteria (Al-Tarawneh, 2004, Bounatirou et al. 2007).

The same findings are reported by Chitra et al. (2011) who showed that the ethanol extract exhibited maximum anti-bacterial activity against *Salmonella enterica* followed by *Staphylococcus aureus* with a diameter of the inhibition zone of 42 mm and 39 mm respectively. This difference may also be related to the species under study.

For all the results, it is recorded that *K. pneumoniae* remains the most sensitive bacterium with respect to all the extracts of plants studied.

On the other hand, some studies show that *T. capitatus* methanol and hexane extracts have no antibacterial activity against Gram-positive bacteria and Gram-negative bacteria or fungi (Mahasneh et al., 1999).

Sokmen et al (2003) report the efficacy of the chloroform extract of *Achilleas* intensive against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*, and the inefficacy of polar fractions.

Our results are in this context and are in line with those of the literature.

Recent work reported by Bekele et al. (2015) mention that the chloroform extract of *Thymus schimperi* has a very good antibacterial activity with respect to the aqueous extract. Similarly, Chitra et al. (2011) reports that the organic extracts of the leaves *Thymus vulgaris* possess a strong antibacterial activity with respect to the

aqueous extract. Similarly, the results given by Qaralleh (2009) reveal that the ethanolic extract of *Thymus capitatus* is more active than The aqueous extract against the tested bacteria. Some studies have shown that the ethanolic extract of cumin is active against *Escherichia coli* ATCC, *Pseudomonas aeruginosa* ATCC, relative to the crude hydro-methanolic extract of cumin (Ertürk, 2006).

CONCLUSION

The antibacterial effect of the chloroform, ethyl acetate, ethanolic, methanolic and aqueous extracts was evaluated by the well-diffusion method of ten bacterial strains. The results show that only the two chloroformic (F1) and ethanolic (F3) extracts at concentrations 1 and 0.75 g / ml *Thymus willdenowii* exerted an antibacterial effect against all the bacterial strains tested. While the extracts of ethyl acetate, methanol and aqueous are inactive. *Pneumoniae* is the only bacterium which exhibits certain sensitivity towards all the extracts of the plants tested.

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