

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *THYMUS VULGARIS L.*

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Thyme (*Thymus vulgaris*) is a Lamiaceae used since antiquity in traditional medicine, recognized by its therapeutic virtues. In this context the present work concerns a phytochemistry study aimed to perform the extraction, quantification and the separation of different phases containing the major flavonoids and an evaluation of their antioxidant and antibacterial activities. We used the method of Folin Ciocalteu and AlCl₃, respectively, to estimate the total of polyphenols and flavonoids. The main phases containing flavonoids have been obtained following confrontations realized by solvents of increasing polarity. The antioxidant capacity of the majority of flavonoids contained in this plant is evaluated *in vitro* by the method of trapping the free radical DPPH°. The same flavonoids are subjected to screening for their possible antibacterial activities against some human pathogenic bacteria. The content of polyphenols and flavonoids in *Thymus vulgaris* is 9.07 ± 0.002 mg/g TAE of dry extract for polyphenols and 8.56 ± 0.001 mg/g QE of dry extract for flavonoids. The chromatographic and spectral identification of extracts of thyme revealed the presence of six flavonoids characterized by their antioxidant activity in the order of 70%, 58 %, 52 %, 51%, 50% and 5% respectively for quercetin (3OR', 7OR''), luteolin, luteolin (7OR'), apigenin (5OR', 7OR''), kaempferol (3OR', 7OR''), chrysine (7OR') compared to quercetin standard characterized by its percentage of scavenging DPPH° equivalent to 93.05%. The microbiological results showed that the flavonoids isolated act differently on the bacterial species tested. In conclusion, this work shows that the flavonoids of selected plants have a good antioxidant and antibacterial activity, and can be used for medicinal and therapeutic applications.

Key words: *Thymus vulgaris*, phenolic compounds, flavonoids, antioxidant activity, antibacterial activity.

INTRODUCTION

Reactive Oxygen Species (ROS), such as hydroxyl radical, hydrogen peroxide, and superoxide anions, are produced as by-products in aerobic organisms and have been implicated in the pathology of a vast variety of human diseases including cancer, atherosclerosis, diabetic mellitus, hypertension, AIDS and aging. Therefore, antioxidant activity is important in view of the free radical theory of aging and associated diseases (Halliwell and Gutteridge, 1984; Wallace, 1999; Lee

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et al., 2000). Researchers have also reported antimicrobial activity of several medicinal plants because of the emergence of multi-drug resistant (MDR) bacteria, since it is a major cause of treatment failure in many infectious diseases. Thus it is necessary to search for alternative antimicrobial agents. One of the possible strategies towards this objective involves the identification and characterization of bioactive phytochemicals, which have antibacterial activity (Ahanjan *et al.*, 2008) and the green plants which possess the broadest spectrum of synthetic activity and have been the source of many useful compounds. These plants are also recognized by their antioxidant activities, and these antioxidants are compounds that when present in foods at low concentrations, compared with the concentration of an oxidizable substrate, markedly delay or prevent oxidation of the substrate (Cavero *et al.*, 2005).

Thymus vulgaris L. is an important medicinal plant (Al-Bayati, 2008; Bazylko and Strzelecka, 2007; Golmakani and Rezaei, 2008; Jiminez-Arellanes *et al.*, 2006 ; Takeuchi *et al.*, 2004). Belonging to the Lamiaceae family, and which has been used for centuries as spice, home remedy, drug, perfume and insecticide. In medicine, it is used as antispasmolytic, antibacterial, antifungal, secretolytic, expectorant, antiseptic, antihelminthic and antitusive as reported by other authors (Özgüven and Tansi, 1998). In this context the aims of this study were therefore to isolate the principal flavonoids contained in the aqueous and solvent extracts from the leaves of *Thymus vulgaris* and to screen their antioxidant and antibacterial activities.

MATERIAL AND METHODS

Plant material, extraction and separation. *Thymus vulgaris* leaves were collected from Constantine, Algeria in May 2008. The plant material was dried at room temperature and ground in a mortar. One hundred of the plant powder was extracted by the classical method of maceration at room temperature with ethanol/water (30:70). The extract was concentrated under reduced pressure and dissolved in distilled water. The solution was successively partitioned with petroleum ether, diethyl ether, ethyl acetate (EtOAc), and butanone (Merghem *et al.*, 1995) The chromatographic and spectral identification of extracts of thyme revealed the presence of six flavonoids.

Total phenols determination. The total of phenolic compounds is determined by using the Folin ciocalteu reagent (Adesegun *et al.*, 2007). Calibration curve was prepared by mixing ethanolic solution of tannic acid (1 ml; 0.01–0.09 mg/ml) with 5 ml Folin ciocalteu reagent (diluted tenfold) and sodium carbonate (4 ml, 0.7 M). We measured absorbance at 765 nm and drew the calibration curve. One milliliter of ethanolic extract (0.05 mg/ml) was also mixed with the reagents above and after two hours the absorbance was measured to determine total plant phenolic contents.

All determinations were carried out in triplicate. The total content of phenolic compounds in the extract in tannic acid equivalent (TAE) was calculated by the following formula:

$$T = C \cdot V/M$$

where:

T: total content of phenolic compounds, milligram per gram extract, in TAE.

C: the concentration of tannic acid established from the calibration curve, milligram per milliliter.

V: the volume of extract, milliliter.

M: the weight of ethanolic plant extract, gram.

Total flavonoids determination. Aluminium chloride colorimetric method is used for flavonoids determination (Ayoola *et al.*, 2008). To 2 ml of 2% AlCl₃ in ethanol is added 2 ml of the test sample. The UV absorption is measured at 420 nm after one hour at room temperature. Concentration of 0.05 mg/ml sample solution is used while quercetin concentrations of (0.01–0.09) mg/ml are used to obtain a calibration curve. Determinations were performed in triplicates. Total flavonoid contents were obtained from the regression equation of the calibration curve of quercetin ($Y = 0.1085x$, $r^2 = 0.96$).

Free radical scavenging activity determination. We used the stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH[•]) for determination of free radical scavenging activity of the flavonoids isolated from *Thymus vulgaris* (Es-Safi *et al.*, 2007). Briefly, 50 µl of methanolic solution containing the compounds to be tested were added to 5 ml of a 0.004% methanolic solution of DPPH[•]. The studied compounds are tested with MeOH as control and quercetin as antioxidant reference and absorbance at 517 nm is determined after 30 min. The absorbance (A) of the control and samples was measured, and the DPPH[•] scavenging activity in percentage is determined as follows:

$$\text{DPPH}^\bullet \text{ scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}})/ A_{\text{control}}] \times 100$$

The data are presented as mean of triplicate.

Antibacterial activity assay. In this work the antibacterial activity of the isolated flavonoids is determined by the disc diffusion method on Mueller-Hinton Agar (MHA) medium (Dulger and Gonuz, 2004; Parekh and Chanda, 2007; Rota *et al.*, 2008). Two clinical isolates of *Escherichia coli* and *Staphylococcus aureus*, obtained from the bacteriology department of the university hospital of Constantine, Algeria, are used for this study. They were isolated and purified on specific nutrient agar plates and characterized by the use of standard microbiological and biochemical methods. The bacteria mentioned above are incubated at 37°C for 24 h by inoculation into nutrient broth. An inoculum containing 10⁶ bacterial cell/ml is spread on Muller-Hinton Agar plates (1 ml inoculum/plate). The discs injected with extracts are placed on the inoculated agar

by pressing slightly. Petri dishes are placed at 4 °C for 2 h, and are incubated at 37 °C for 24 h. At the end of the period, inhibition zones formed on the medium are evaluated in mm. Studies were performed in triplicate.

RESULTS AND DISCUSSION

Total phenolic and flavonoids contents determination

The amount of total phenolic contents is 9.07 ± 0.002 mg/g TAE of dry material. The flavonoid content in the extract of *Thymus vulgaris* in terms of quercetin equivalent was 8.56 ± 0.001 mg/g QE of dry material. Figure 1 shows that the flavonoids had the highest amount in the studied plant; these compounds, which contain hydroxyls, are responsible for the radical scavenging effect in plants (Middleton *et al.*, 2000; Amić *et al.*, 2003).

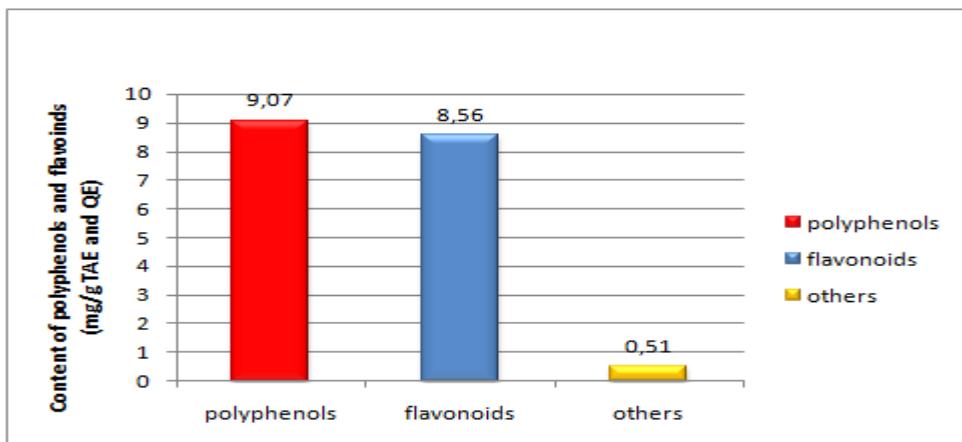


Fig. 1. Phenolic and flavonoids contents in the plant extract studied (*Thymus vulgaris*).

Antioxidant activity of extracts

The stable free radical DPPH° method is an easy, rapid, and sensitive way to survey the antioxidant activity of specific compounds or plant extracts (Ebrahimzadeh *et al.*, 2008).

The isolated flavonoids from thyme are tested for their antioxidant scavenging effects on DPPH° radical and we compared their activity to a quercetin standard used as antioxidant reference. The results obtained are given in Figure 2 and expressed as the percentage of scavenging activity of DPPH°. The result of our experiment demonstrated that the quercetin (3OR', 7OR'') possesses the higher antioxidant activity, luteolin, luteolin (7OR'), apigenin (5OR', 7OR''), and

kaempferol (3OR', 7OR'') have a moderate activity while the chrysin (7OR') has a low radical scavenging activity.

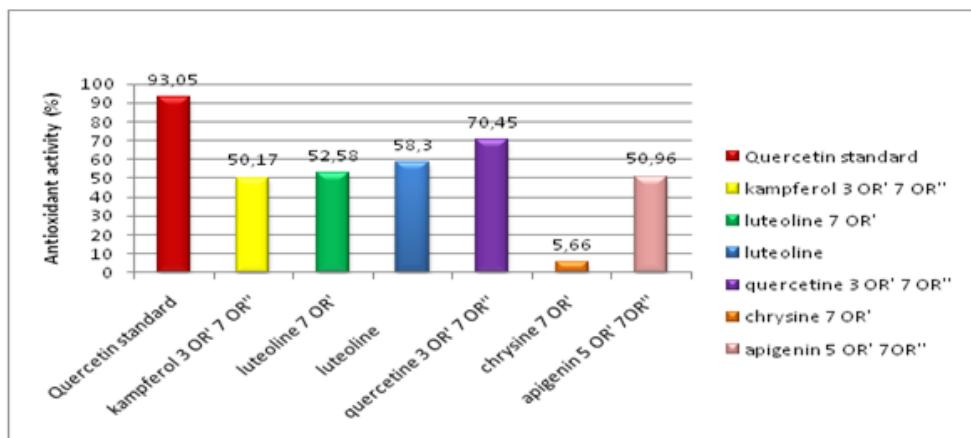


Fig. 2. Percentage of the scavenging activity of DPPH° by the flavonoids isolated from *Thymus vulgaris*.

A preliminary phytochemical analysis of the ethanolic extract of thyme revealed the presence of phenolic compounds (Cowan, 1999; Takeuchi *et al.*, 2004) and flavonoids (Takeuchi *et al.*, 2004; Bazylco and Strzelecka, 2007), to which are attributed many of the antioxidant properties, due to their hydrogen donation ability, and their structural requirement considered to be essential for effective radical scavenging, it has been reported that this activity may result from:

The presence of a 3', 4'-dihydroxy, *i.e.*, a *o*-dihydroxy group (catechol structure) in the B ring, possessing electron donating properties and being a radical target.

- The 3-OH moiety of the C ring is also beneficial for the antioxidant activity of flavonoids.
- The C2 = C3 double bond conjugated with a 4-keto group, which is responsible for electron delocalization from the B ring, enhances further the radical-scavenging capacity.
- The presence of both 3-OH and 5-OH groups in combination with a 4-carbonyl function and C2 = C3 double bond.
- The presence of hydroxyl substituents in a catechol structure on the A-ring is able to compensate the absence of the *o*-dihydroxy structure in the B-ring, and became a larger determinate of flavonoid antiradical activity (Amić *et al.*, 2003).

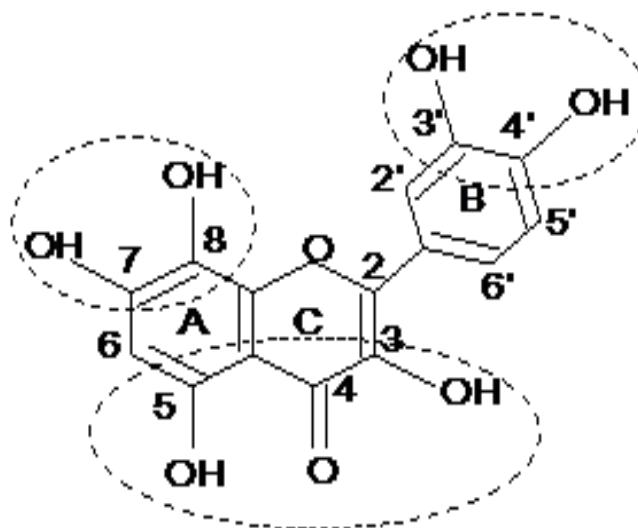


Fig. 3. Structural features of flavonoids with a high radical scavenging activity.

Antibacterial activity determination

The antibacterial activity of our products is estimated in terms of diameter of zone of inhibition around disks containing the flavonoid products to be tested against *Escherichia coli*, *Staphylococcus aureus* after 24 hours of incubation at adequate temperature of 37°C.

The results of the diameters of the inhibition zones reveal that *Escherichia coli* seems sensitive towards the tested flavonoids, which develop important zones of inhibition towards *Staphylococcus aureus* whose the diameters of the inhibition zones vary between 8–18 mm for *Escherichia coli*, and 10–15 mm for *Staphylococcus aureus*. The sensitivity of *Escherichia coli* and *Staphylococcus aureus* reflects the antibacterial action of flavonoids. In fact, this sensitivity is related to the number of free hydroxyls which shows that the least hydroxylated flavonoids are most active, for example the chrysin 7 OR' (which has only one free OH at the carbon 5) develops zones of inhibition equivalent to 15 mm for *Staphylococcus aureus* and 18 mm for *Escherichia coli*.

Cowan (1999) assumed that flavonoids lacking the free hydroxyl groups have more antimicrobial activity compared to those who are filled, which leads to an increase of the chemical affinity for membrane lipids, thus we can assume that the target microbial of these flavonoids tested is the cytoplasmic membrane.

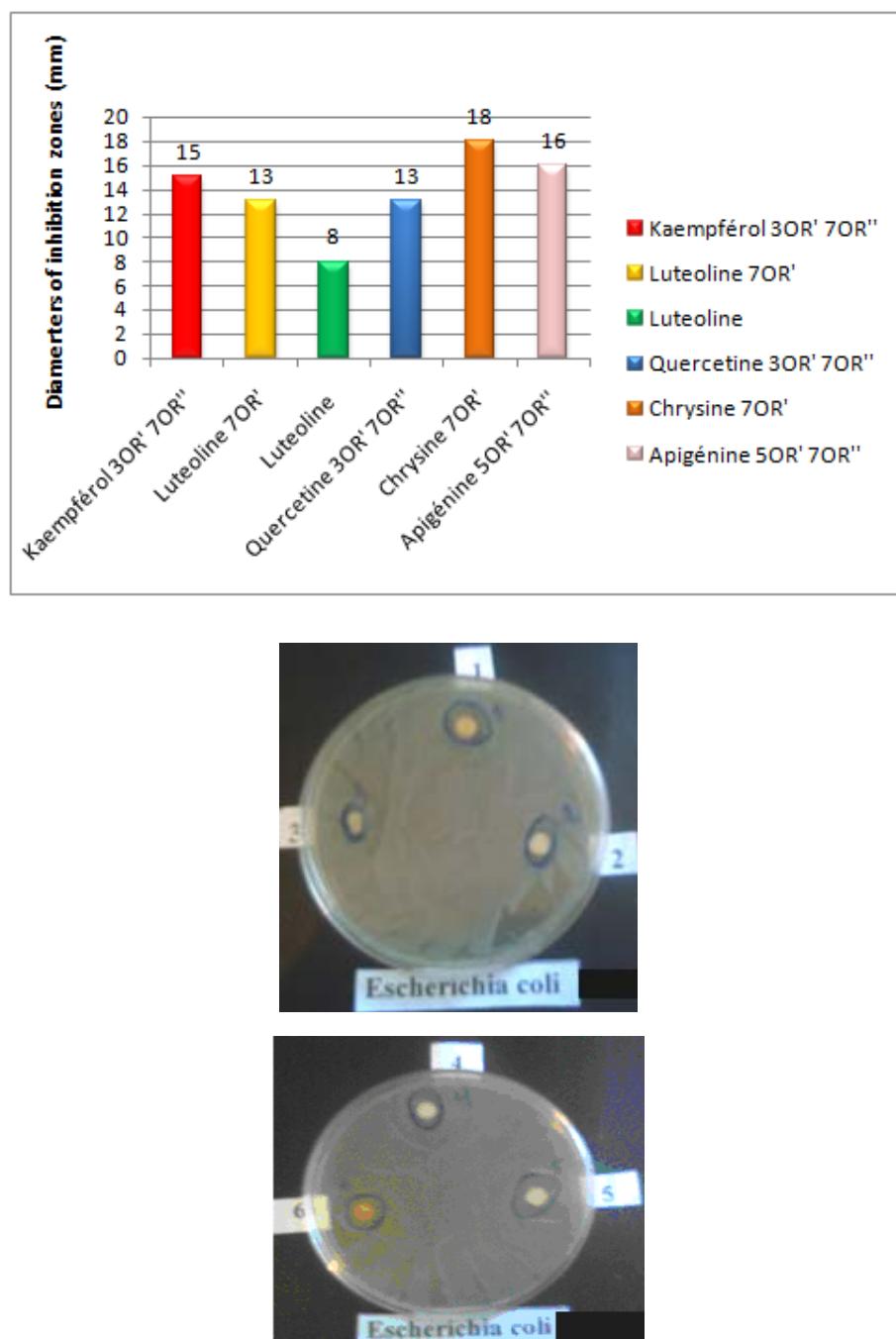


Fig. 4. Diameters of inhibition zones (mm) of the main flavonoids isolated from *Thymus vulgaris* against *Escherichia coli*.

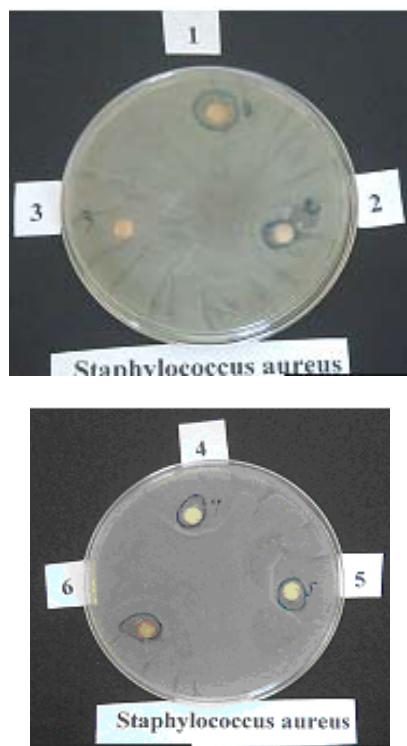
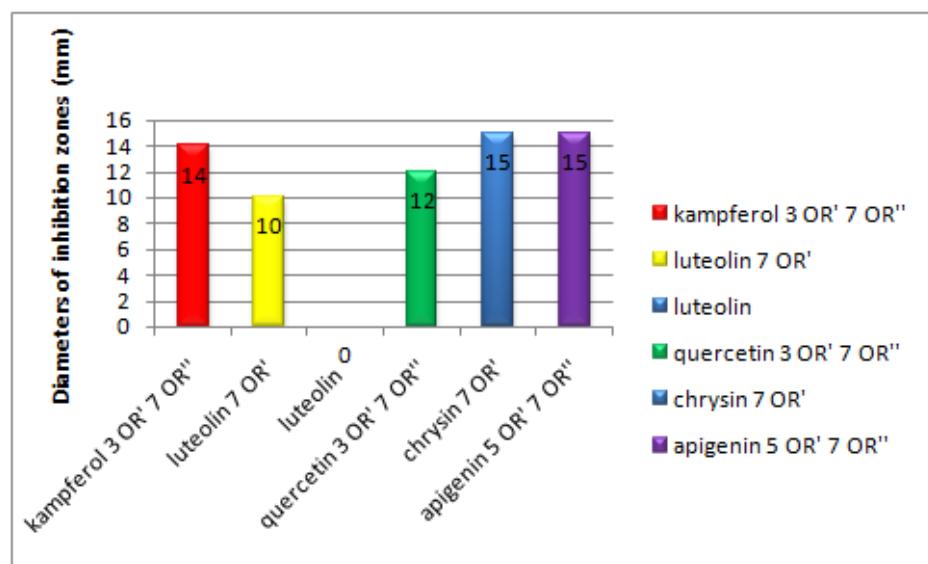


Fig. 5. Diameters of inhibition zones (mm) of the main flavonoids isolated from *Thymus vulgaris* against *Staphylococcus aureus*.

CONCLUSION

Thymus vulgaris is an important source of phenolic compounds, and the result of the present study showed that the extract of this plant contains a high amount of flavonoids, exhibited a great antioxidant and antibacterial activity. In this context, the thyme can be used as an easily accessible source of natural antioxidants and antibiotics in commercial food products and drugs. In future work we will propose:

- To use new techniques such (**HPLC, RMN, MS...**) that we provide to determine a more precise structural identification of molecules;
- To develop antiradical and antibacterial drugs based on plants.

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