Medicinal and Aromatic Plant Research Journal
Vol. 1(1), pp. 5-11, September 2013
Research Paper

Antioxidant and antibacterial activities of *Thymus vulgaris* L.

Zeghad Nadia* • Merghem Rachid

Laboratory of the Development and Utilization of Genetic Resources of Plants, Faculty of Natural Sciences and Life, University Constantine I, Algeria.

*Corresponding author. E-mail: zeghadsofia@yahoo.fr.

Accepted 5th July, 2013

Abstract. Thyme (*Thymus vulgaris*) belongs to the Lamiaceae family, and has been used since antiquity in traditional medicine. It is recognized by its therapeutic virtues. In this context, the objective of the present paper is to perform the extraction, quantification and 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 $AlCl_3$, 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 of 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 *T. vulgaris* is 9.07 ± 0.002 mg/g Tannic Acid Equivalent of dry extract for polyphenols and 8.56 ±0.001 mg/g Quercetin Equivalent 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'') (were R' and R'' are unknown radicals), luteolin, luteolin (7OR'), apignin (5OR', 7OR''), kaempfrol (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 the selected plants have good antioxidant and antibacterial activity, and can be used for medicinal and therapeutic applications.

Keywords: *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 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). Green plants posses the broadest spectrum of synthetic activity and have been the source of many useful compounds. These plants are also recognized by their antioxidant activities, which 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 are recurrent flavonoids, these metabolites are a group of pigments contained in plants and they are responsible for flower and fruit coloration. Flavonoids are present in dietary fruit and vegetables and responsible for many biological properties included antioxidant activities (Tripoli, 2007).

T. 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) which belongs to the Lamiaceae family; it has been used for centuries as spice, home remedy, drug, perfume and insecticide. In medicine, it is used as antispasmolytic, antibacterial, antifungal, secrotolytic, expectorant, antiseptic, antlelmintic and antitusive as reported by other authors (Özgüven and Tansi, 1998). In this context, the aims of this study were therefore to isolate principal flavonoids contining in the aqueous and solvent extracts from the leaves of *T. vulgaris* and to screen their antioxidant and antibacterial activities.

MATERIALS AND METHODS

Plant material, extraction and separation

T. vulgaris leaves are collected from Constantine, Algeria in May 2008. The plant material was dried at room temperature and ground in a mortar. 100 g of the plant powder was extracted by the classical method of maceration at room temperature with ethanol/water (30:70). The ethanolic extract was concentrated under reduced pressure and the dry residue was dissolved in 100 ml of boiling distilled water. After filtration using filter paper, the solution was partitioned successively with petroleum ether, diethylic ether, ethyl acetate (EtOAc), and methyl ethyl cetone (MEC) (Merghem et al., 1995), the chromatographic and spectral identification of extracts of thyme revealed the presence of six flavonoids.

Total phenols determination

The total content 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 to 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 2 h 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 (Tannic Acid Equivalent) was calculated by the following formula:

$$T = C.V/M$$

where:
T: total content of phenolic compounds, milligram per gram extract, in Tannic Acid Equivalent.
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). Two milliliters of 2% $AlCl_3$ in ethanol is added to 2 ml of the test sample. The UV absorption is measured at 420 nm after 1 h at room temperature. Concentration of 0.05 mg/ml sample solution is used while quercetin concentrations of 0.01 to 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 *T. 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 methanol 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 follow:

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

The data are presented as mean of triplicate.

Antibacterial activity assay

In this work, the antibacterial activity of the isolated flavonoids are 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* ATCC

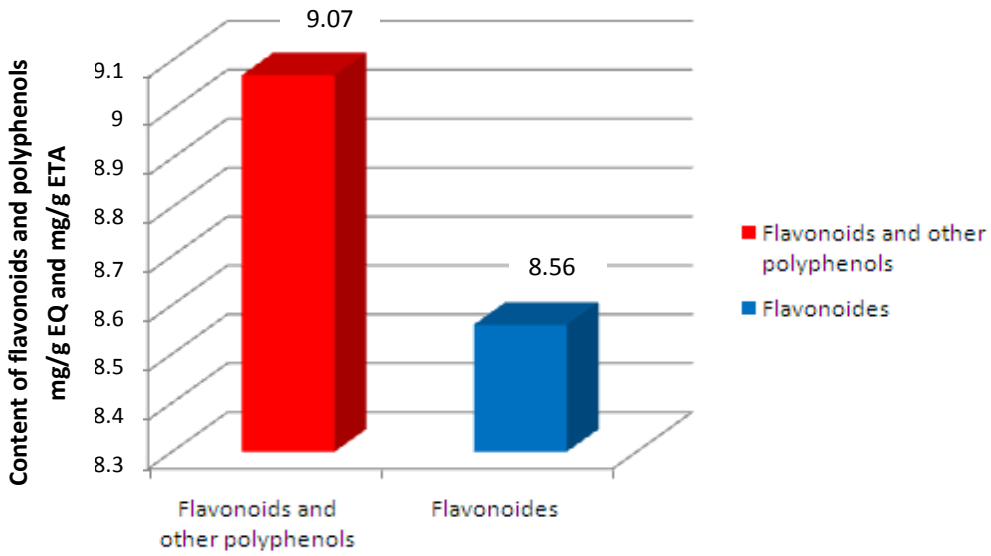

Figure 1. Phenolic and flavonoids contents in the studied plant extract (*Thymus vulgaris*).

Table 1. Percentage of the scavenging activity of DPPH° by the flavonoids isolated from *Thymus vulgaris*.

	Quercetin standard	Kaempferol 3OR' 7OR''	Luteolin 7OR'	Luteolin	Quercetin 3OR' 7OR''	Chrysin 7OR'	Apigenin 5OR' 7OR''
Antioxidant activity (%)	93.05 ± 0.01	50.17 ± 0. 08	52.58 ± 0.02	58.3 ± 0.03	70.45 ± 0.04	5.66 ± 0.02	50.96 ± 0.07

Data are expressed as means ± standard deviation (n = 3).

25922 and *Staphylococcus aureus* ATCC 25923 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 10^6 bacterial cell/ml is spread on Muller-Hinton Agar plates (1 ml inoculums/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 to facilitate the dissemination of extract on the culture medium, and then incubated at 37°C for 24 h to ensure the growth of bacterial strains tested. At the end of the period, inhibition zones formed on the medium are evaluated in mm. Studies were performed in triplicate, and quercetin is used as positive control.

RESULTS AND DISCUSSION

Total phenolic and flavonoids contents determination

The amount of total phenolic contents is 9.07 ± 0.002 mg/g Tannic Acid Equivalent of dry material. The flavonoid contents in the extract of *T. vulgaris* in terms of quercetin equivalent were 8.56 ± 0.001 mg/g Quercetin Equivalent of dry material. Figure 1 shows that the flavonoids present an important amount in the studied plant; these compounds which contain hydroxyls are responsible for the radical scavenging effect in the plants (Middleton et al., 2000; Amić et al., 2003).

Antioxidant activity of the extracts

The stable free radical DPPH° method is an easy, rapid and sensitive way to survey the antioxidant activity of a 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 (Table 1, Figure 2) and expressed as the percentage of the scavenging activity of DPPH°. The result of our experiment demonstrated that quercetin (3OR', 7OR'') (R', R'' unknown radicals) possesses high antioxidant activity, luteolin, luteolin (7OR'), apigenin (5OR', 7OR'') and kaempferol (3OR', 7OR'') have a moderate activity, while the chrysin (7OR') has low radical scavenging activity.

A preliminary phytochemical analysis of the ethanolic extract of thyme revealed the presence of phenolic compounds (Cowan, 1999; Takeuchi et al., 2004) and

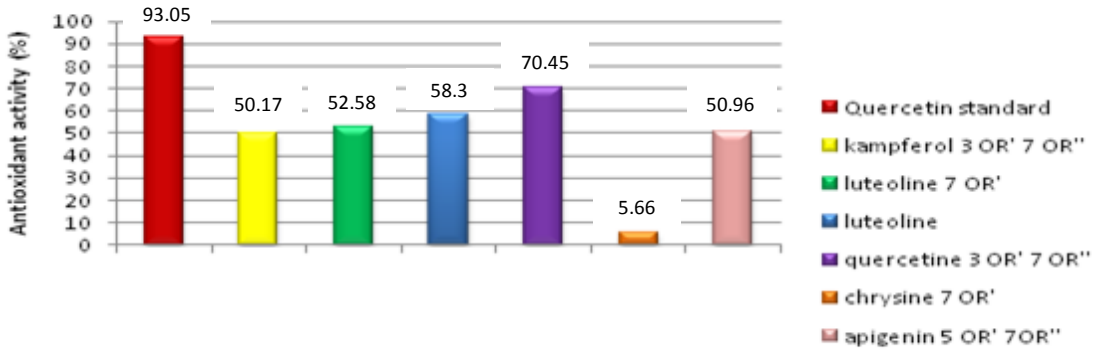

Figure 2. Percentage of the scavenging activity of DPPH° by the flavonoids isolated from *Thymus vulgaris*.

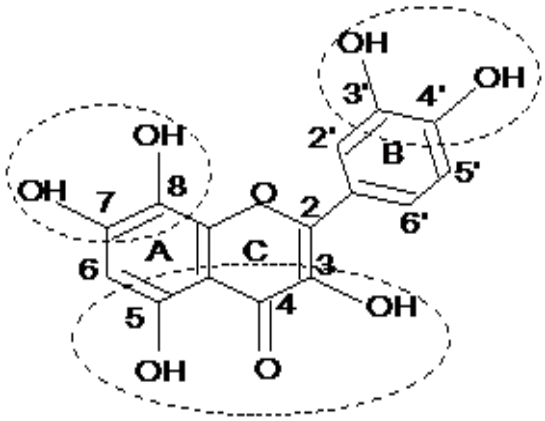

Figure 3. Structural features of flavonoids with a high radical scavenging activity.

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:

a) 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.
b) The 3-OH moiety of the C ring is also beneficial for the antioxidant activity of flavonoids.
c) 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.
d) The presence of both 3-OH and 5-OH groups in combination with a 4-carbonyl function and C2-C3 double bond.
e) The presence of hydroxyl substituents in a catechol structure on the A-ring, which are able to compensate the absence of the *o*-dihydroxy structure in the B-ring, and becomes a larger determinate of flavonoid antiradical activity (Amić et al., 2003). (Figure 3).

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 *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 after 24 h of incubation at adequate temperature of 37°C (Table 2, Figure 4).

The results of the diameters of the inhibition zones reveal that *E. coli* ATCC 25922 seems sensitive towards the tested flavonoides, where these flavonoides develop important zones of inhibition towards *S. aureus* ATCC 25923, with the diameters of the inhibition zones vary between 8 and 18 mm for *E. coli* ATCC 25922, and of 10 to 15 mm for *S. aureus* ATCC 25923. The sensibility of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 reflects the antibacterial action of flavonoids. In fact, this sensitivity is related to the number of free hydroxyl which shows that the least hydroxylated flavonoids are most active, for example the chrysin 7OR' (which has only one free OH at the carbon 5) develops zones of inhibition equivalent to 15 mm for *S. aureus* ATCC 25923 and 18 mm for *E. coli* ATCC 25922 (Table 3, Figure 5).

Cowan (1999) assumed that flavonoids lacking the

Table 2. Diameters of inhibition zones (mm) of the main flavonoids isolated from *Thymus vulgaris* against *Escherichia coli ATCC 25922.*

	Quercetin standard	Kaempferol 3OR' 7OR''	Luteolin 7OR'	Luteolin	Quercetin 3OR' 7OR''	Chrysin 7 OR'	Apigenin 5OR' 7OR''
Diameters of inhibition zones (mm)	0 ± 0	15 ± 0.18	13 ± 0.22	8 ± 0.33	13 ± 0.04	18 ± 0.22	16 ± 0.17

Data are expressed as means ± standard deviation (n = 3).

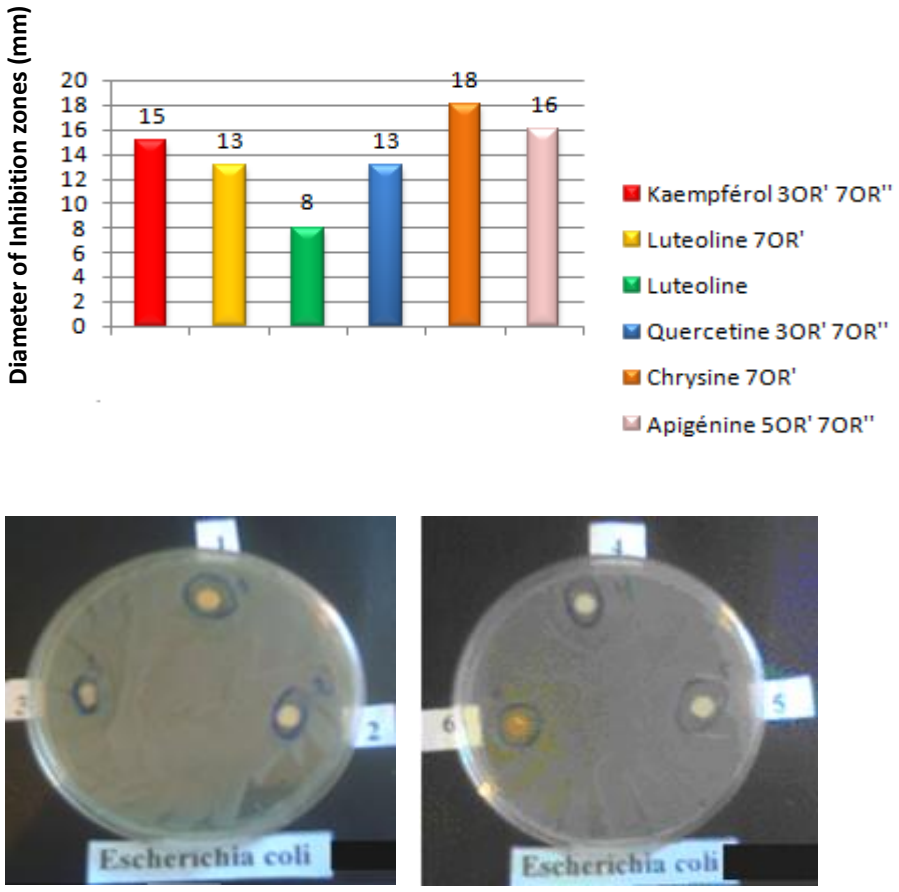

Figure 4. Diameters of inhibition zones (mm) of the main flavonoids isolated from *Thymus vulgaris* against *Escherichia coli* ATCC 25922.

Table 3. Diameters of inhibition zones (mm) of the main flavonoids isolated from *Thymus vulgaris* against *Staphylococcus aureus* ATCC 25923.

	Kaempferol 3OR' 7OR''	Luteolin 7OR'	Luteolin	Quercetin 3OR' 7OR''	Chrysin 7 OR'	Apigenin 5OR' 7OR''
Diameters of inhibition zones (mm)	14 ± 0. 25	10 ± 0.14	0 ± 0.0	12 ± 0.46	15 ± 0.06	15 ± 0.06

Data are expressed as means ± standard deviation (n = 3).

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.

Conclusion

T. vulgaris is an important source of phenolic compounds. The result of the present study showed that the extract of this plant contain high amount of flavonoids,

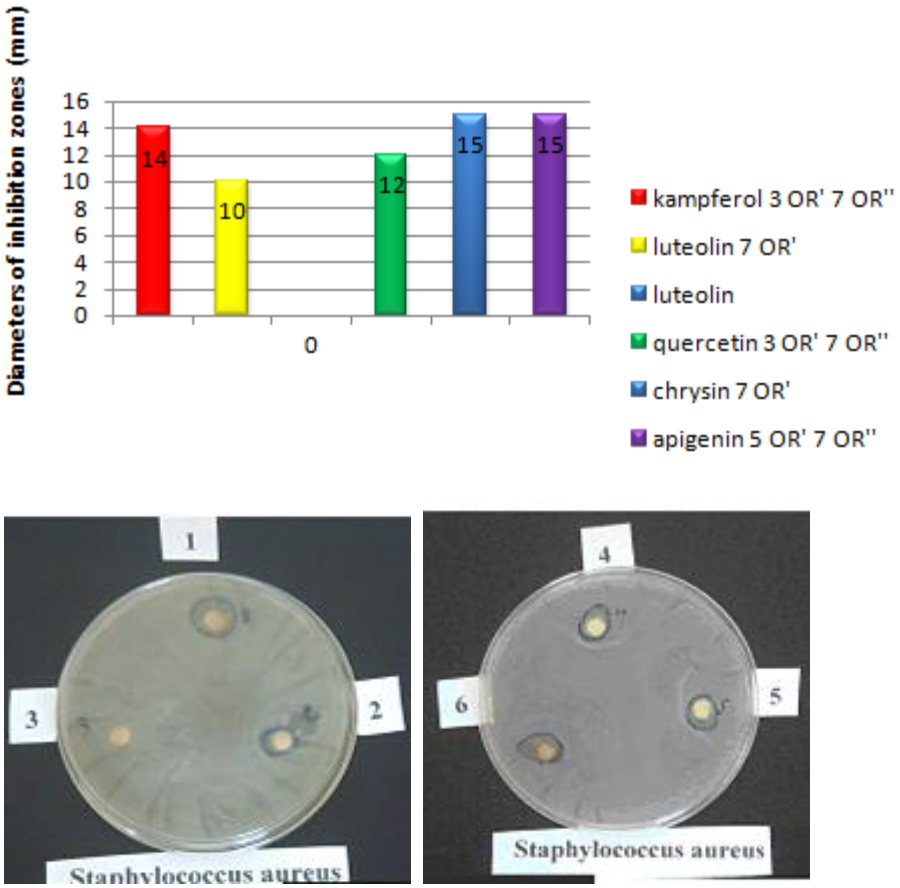

Figure 5. Diameters of inhibition zones (mm) of the main flavonoids isolated from *Thymus vulgaris* against *Staphylococcus aureus* ATCC 25923.

and exhibited a great antioxidant and antibacterial activity. In this context, thyme can be used as an easily accessible source of natural antioxidants and antibiotics in commercial food products and drugs.

RECOMMENDATION

In future work, we will propose:

i) The use of new techniques such HPLC, RMN and MS, to determine a more precise structural identification of molecules
ii) The development of antiradical and antibacterial drugs based on plants.

REFERENCES

Adesegun SA, Fajana A, Orabueze CI, Coker HAB (2007). Evaluation of antioxidant propreties of Phaulopsis fascisepala C B Cl (Acanthaceae). J. Evidence based complementary and altern. med. 6(2):227-231.

Ahanjan M, Mohana DC, Raveesha KA (2008). Antibacterial activity of Asperugo procumbens L. against some human pathogenic bacteria. Afr. J. Microbial. Res. 2:138-140.

Al-Bayati FA (2008). Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts.J. J. Ethnopharmacol. 166(3):403-406.

Amić D, Davidović-Amić D, Bešlo D, Trinajstić N (2003). Structure–Radical scavenging activity relashionships of flavonoids. J. *Croat. Chem. Acta.* 76(1):55-61.

Ayoola GA, Ipav SS, Solidiya MO, Adepoju-Bello AA, Coker HAB, Odugbemi TO (2008). Phytochemical screening and free radical scavenging activities of the fruits and leaves of Allanblackia floribunda oliv (Guttiferae). J. Int. J. health Res. 1(2):81-93.

Bazylko A, Strzelecka H (2007). A HPTLC densitometric determination of luteolin in *Thymus vulgaris* and its extracts. J. *Fitotherapia.* 78:391-395.

Cavero S, Jaime L, Martin-Ălvarez PJ, Señoráns FJ, Reglero G, Ibañez E (2005). *In vitro* antioxidant analysis of supercritical fluid extracts from rosmary (*Rosmarinus officinalis* L). J. *Eur. food Res. technol.* 221:478-486.

Cowan MM (1999). Plant products as antimicrobial agents. J. *Clin. Microbial. Rev*. 12(4):564-570.

Dulger B, Gonuz A (2004). Antimicrobial activity of some Turkish medicinal plants. J. Pak. J. Boil. Sci. 7(9):1559-1562.

Ebrahimzadeh MA, Pourmmorad F, Hafezi S (2008). Antioxidant activities of Iranian corn silk, *Turk. J. Biol.* 32:43-49.

Es-Safi N E, Kollmann I, Khlifi S, Ducrot PH (2007). Antioxidants effect of compounds isolated from *Globularia alypum* L Structure-activity relationship, *LWT-Food Sci. TechnoL.*. 40:1246-1252.

Golmakani MT, Rezaei K (2008). Comparaison of microwave-assisted hydrodistillation with the traditional hydrodistillation method in the extraction of essential oils from *Thymus vulgaris* L, Food chem. 109:925-930.

Halliwell B, Gutteridge JM (1984). Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy, *Lancet.* 8391:1396-1397.

Jiménez-Arellanes A, Martinez R, Garcia R, León-Diaz R, Aluna–Herrera J, Molina –Salinas G Said-Fernández S (2006). *Thymus vulgaris* as a potential source of antituberculosis compounds, *Pharmacologyonline.* 3:569-574.

Lee S, Suh S, Kim S (2000). Protective effects of the green tea polyphenol (-)-epigallocatechin gallate against hippocampal neuronal damage after transient global ischemia in gerbils, *Neurosci. Lett.* 287:191-194.

Merghem R, Jay M, Viricel MR, Bayet C, Voirin B (1995). Five 8-C benzylated flavonoids from *Thymus hirtus* (Labiateae), *Phytochemistry.* 38(3):637-640.

Middleton E, Kandaswami C, Theoharides TC (2000). The effects of plants flavonoids on mammalian cells: Implication for inflammation, heart disease, and cancer, Phamacol. Rev. 52(4):673-751.

Özgüven M, Tansi S (1998). Drug yield and essential oil of *Thymus* vulgaris L as in influenced by ecological and ontogenetical variation, Turk. J. agric. for. 22:537-542.

Parekh J, Chanda SV (2007). *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plant, *Turk. J. Biol.* 31:53-58.

Rota MC, Herrera A, Martinez RM, Sotomayor JA, Jordán MJ (2000). Antimicrobial activity and chemical composition of *Thymus vulgaris*, Thymus zygis and Thymus hyemalis essential oils, Food control. 19:681-687.

Takeuchi H, Lu ZG, Yu O (2007). Distinct crucial roles of flavonoids during legume nodulation, *Trends in Plant Sci.* 12(7):282-283.

Tripoli E, La Guardia M, Giammanco S, Di Majo D, Giammanco M (2007). Citrus flavonoids : Molecular structure, biological activity and nutritional propreties : A review, Food Chem. 104:466-479.

Wallace DC (1999). Mitochondrial diseases in man and mouse, *Science.* 283:1482-1488.