Journal of Enzyme Inhibition and Medicinal Chemistry

http://informahealthcare.com/enz
ISSN: 1475-6366 (print), 1475-6374 (electronic)

J Enzyme Inhib Med Chem, Early Online: 1–6
 DOI: 10.3109/14756366.2014.930453

informa
healthcare

ORIGINAL ARTICLE

Antibacterial, antioxidant and hypoglycaemic effects of *Thymus capitatus* (L.) Hoffmanns. et Link leaves' fractions

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Abstract

The aim of this study was to examine the bioactivity of the methanol fraction (MF) and *n*-hexane fraction (HF) of *Thymus capitatus* leaves in relation to their constituents analysed by gas chromatography and gas chromatography-mass spectrometry. The effects of *T. capitatus* on the growth of pathogenic bacteria associated with respiratory diseases (13 gram-positive and 4 gram-negative) were determined using a microdilution method. The MF was particularly effective on *Streptococcus pneumoniae* and *Moraxella catarrhalis*. The antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ferric-reducing antioxidant power and β-carotene bleaching assays. A strong activity using β-carotene bleaching test was observed with the MF (IC_{50} of 0.7 μg/mL after 30 min of incubation). In the hypoglycaemic test, a selective α-amylase inhibitory activity was detected with the HF begging the most active (IC_{50} of 422.5 μg/mL). *T. capitatus* may represent a source of natural bioactive compounds.

Keywords

Antibacterial properties, antioxidant, GC-MS analysis, hypoglycaemic, *Thymus capitatus*

History

Received 27 January 2014
Revised 20 May 2014
Accepted 29 May 2014
Published online 16 July 2014

Introduction

Thymus capitatus (L.) Hoffmanns. et Link (Lamiaceae) is a shrub species high 20–150 cm, very aromatic, native of the Mediterranean and very popular in Italy. The plant has a strong root, is woody and is much branched with branches covered by numerous trichomes of coating that give it a whitish colour. The leaves are small, sessile, linear, acute apex and grey-green. Inflorescences, oblong-conical, have flowers with a corolla ranging from pink to red; the fruit is a brown ''tetrachenio''[1,2].

Thymus species are used in traditional medicine as tonic, antiseptic, antitussive and carminative as well as for treating colds and in pharmaceutical, cosmetic and perfume industry for preservation of several food products or as spice[1,3,4]. Different studies on *Thymus* species, and in particular of the essential oil, demonstrated the antibacterial, antihypertensive, antioxidant, hepatoprotective, antitumor and hypoglycaemic activities[5–9]. Moreover, in veterinary medicine more than 280 plant species, mainly in the form of decoctions or dietary supplements, have been used for the treatment of various diseases such as gastrointestinal, respiratory and skin diseases, wounds or inflammation. *Thymus* spp. could represent a valid alternative to chemical products also on organic farms, where the EU Regulation 1804/99 has banned the use of antibiotic synthesis[10]. Therefore, the use of herbal products in animal farms offers many important advantages such as absence of chemical residues in meat, and a decrease of costs.

Worldwide infectious diseases are still the leading cause of death, especially in developing countries, claiming millions of lives yearly despite the enormous improvements made in human healthcare[11]. Respiratory diseases are second to cardiovascular conditions in terms of mortality, incidence, prevalence and costs. Respiratory diseases range from acute infections, such as pneumonia and bronchitis, to chronic conditions such as asthma and chronic obstructive pulmonary disease and include unspecific symptoms such as dyspnea. The increasing recognition and importance of antibacterial infections, the difficulties encountered in their treatment and the increase in resistance have stimulated the search for therapeutic alternatives. Medicinal plants have provided remedies for chronic and moderate health troubles and sometimes for acute and severe disorders[12].

It is commonly accepted that in a situation of oxidative stress, reactive oxygen species (ROS) are generated. The ROS plays an important role in the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts and inflammation[13]. The burst of ROS is considered one of the key factors also in the development of diabetes[14].

Diabetes mellitus is a metabolic disorder which is characterized by increased blood glucose levels with conflict in carbohydrate, protein and fat metabolism, thus leading to absolute

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or relative lack of insulin secretion. The prevalence of this disorder is increased globally and likely to mount up to 80 million patients by 2030, with India estimated to have the leading number of diabetic cases[15]. Type 2 diabetes is the most important hazard to human health because of its growing prevalence and complications. One therapeutic approach to treat the early stage of diabetes is to decrease postprandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. Pancreatic α-amylase stimulates in the digestive system the first step in hydrolysis of starch to a mixture of small oligosaccharides that are further degraded by α-glucosidase into glucose. Degradation of this dietary starch leads to elevated postprandial hyperglycemia[16].

With this background information, the current study was intended to assess the chemical composition of *T. capitatus* leaves' fractions and the antimicrobial activity of these fractions against *Str. pneumoniae*, *Haemophylus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus* according to Clinical and Laboratory Standards Institute procedure (CLSI, 2008)[17]. The same bacteria may be responsible for respiratory diseases in several animal species (*Sta. aureus* in horse and *Str. pneumoniae* in guinea pig). *T. capitatus* fractions were also investigated for their antiradical and antioxidant activities by four *in vitro* methods (DPPH, ABTS, FRAP and β-carotene bleaching tests) and for their potential hypoglycaemic effects (the inhibitory activity of carbohydrate-hydrolysing enzymes α-amylase and α-glucosidase). The relationships between chemical composition and bioactivity were also discussed.

Materials and methods

Plant material

The leaves of *T. capitatus* were collected in Portopalo, Siracusa (Sicily, Italy) in May 2012 and authenticated by Prof. L. Iauk, University of Catania (Italy). A voucher specimen (no. 05/12) has been retained at Department of Bio-Medical Sciences, University of Catania.

Chemicals and reagents

Methanol, ethanol, *n*-hexane, dimethyl sulfoxide (DMSO), $FeSO_4$ thin layer chromatography (TLC) plates were purchased from VWR (Milan, Italy). β-Carotene, anhydrous sodium sulphate, ascorbic acid, propyl gallate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid, Tween 20, sodium phosphate buffer, sodium potassium tartrate tetrahydrate, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) solution, acetate buffer, Trolox, α-amylase from porcine pancreas (EC 3.2.1.1), α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), acarbose, maltose, sodium acetate, sodium potassium tartrate, 3,5-dinitrosalicylic acid, *o*-dianisidine colour reagent (DIAN), peroxidase glucose oxidase (PGO) enzyme solution were purchased from Sigma-Aldrich S.p.a. (Milan, Italy).

Bacterial assay

The identification of bacteria isolated has recourse to the use of catalase (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom); oxidase test (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom), crystal violet, Gram's iodine, 95% ethyl alcohol and safranin (Gram staining); coagulase test (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom); sugar fermentation test and VITEK® 2 Compact bioMérieux Italia Spa (Florence, Italy).

Extraction procedure

The leaves (51.45 g) of *T. capitatus* were dried, powdered and extracted with methanol for 24 h through maceration. Methanol solutions were concentrated and dried to obtain 0.350 g of total extract (yield 0.68%). This extract was solubilised with water and partitioned with $CHCl_3$. After evaporation of the chloroform, the residue was solubilised by methanol and extracted with *n*-hexane. The *n*-hexane solutions were combined and dried to obtain the *n*-hexane fraction (HF). The same procedure was applied to the methanol solution in order to obtain the methanol fraction (MF).

Thin layer chromatography

T. capitatus MF and HF were analysed by using TLC using predetermined amounts of thymol and carvacrol as standards. TLC plates were performed on Silica Gel 60 F254, using as mobile phase $CHCl_3$-toluene (75:25). The plates were examined in ultraviolet light (UV) at 254 nm and then sprayed with vanillin-H_2SO_4.

Gas chromatography/mass spectrometry and gas chromatography analysis

T. capitatus samples were analysed by using a Hewlett-Packard 6890 gas chromatograph equipped with an HP-5 MS capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) and interfaced with a Hewlett-Packard 5973 Mass Selective (Agilent, Milan, Italy)[18]. Ionization was performed in electron impact mode (EI, 70 eV). The analytical conditions were as follows: oven temperature was 5 min isothermal at 50 °C and then 50–250 °C at a rate of 5 °C/min and then held isothermal for 10 min. The injector and detector temperatures were 250 and 280 °C, respectively. Constituents were tentatively identified by gas chromatography (GC) by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratory. Further tentative identification was made by comparison of their mass spectra with those stored in Wiley 138 and NIST 98 libraries. GC analyses were performed on a Shimadzu GC17A gas chromatograph (Shimadzu, Milan, Italy) equipped with a flame ionization detector and controlled by Borwin Software. An SE-52 capillary column (5% phenyl-methylpolysiloxane, 30 m with an internal diameter of 0.25 mm and a film thickness of 0.25 µm) was used with nitrogen as the carrier gas. GC oven temperature and conditions were as described above. The quantitative determinations were carried out by peak area normalization measurements using an external standard method.

Antibacterial activity assay

A total of 17 bacterial isolates (13 gram-positive and 4 gram-negative strains) were included in this study (Table 2). Standard strains of the most common pathogenic bacterial species, such as such as *Str. pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 49247, *M. catarrhalis* ATCC 25238 and *Sta. aureus* ATCC 29213, were used. Moreover, strains from respiratory tract infections, belonging to the bacterial collections of Department of Bio-Medical Sciences, Microbiology Section, University of Catania, and characterized by antibiotic susceptibility profiles of variables (penicillin and/or erythromycin-resistant *Str. pneumonia*, erythromycin-sensitive/resistant *Str. pyogenes*, ampicillin-resistant *H. influenzae* and methicillin-resistant *Sta. aureus*) were used.

The bacterial strains were seeded on tryptic soy agar and incubated aerobically at a temperature of 37 °C for 24 h, and the yeasts were seeded on Sabouraud dextrose agar at 30 °C for 48 h.

Antibacterial assays were performed using a microdilution method according to CLSI[17]. Aliquots of *T. capitatus* fractions were dissolved in DMSO (0.5%), diluted in Mueller Hinton broth and used to assess the antibacterial activity *in vitro* by the method of microdilution broth in order to obtain concentrations of extract of 31.25, 62.5, 125, 250, 500, 1000 µg/mL. The experiments were carried out in triplicate.

DPPH assay

The radical scavenging activity of *T. capitatus* fractions was determined according to the technique previously reported with some modifications[19]. An aliquot (1.5 mL) of 0.25 mM DPPH solution in ethanol was added to 12 µL of *T. capitatus* fractions in ethanol at different concentrations (31.3, 62.5, 125, 250, 500 and 1000 mg/mL). The mixture was shaken and kept in the dark at room temperature for 30 min. The bleaching of DPPH was determined by measuring the absorbance at 517 nm. All tests were run in triplicate and the mean values calculated. Ascorbic acid was used as a positive control. Results were expressed as IC_{50} values (µg/mL). The DPPH radical scavenging capacity (RSC) was calculated according to the following equation: $RSC = [(A_0 - A_1/A_0) \times 100]$, where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract.

ABTS assay

ABTS assay was based on the method of Re et al.[20] with slight modifications. ABTS radical cation ($ABTS^+$) was produced by the reaction of a 7 mM ABTS solution with 2.45 mM potassium persulphate. The mixture was stored in the dark at room temperature for 12 h before use. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. After addition of 25 µL of samples in ethanol (5–80 mg/mL) or Trolox standard to 2 mL of diluted $ABTS^+$ solution, absorbance was measured at 734 nm. The decrease in absorption was used for calculating Trolox equivalent antioxidant capacity values. A standard curve was prepared by measuring the reduction in absorbance of the $ABTS^{\bullet+}$ solution at different concentrations of Trolox. Appropriate blank measurements were carried out, and the values were recorded. Results were expressed as IC_{50} values (µg/mL). Ascorbic acid was used as positive control.

Ferric-reducing antioxidant power assay

The ferric-reducing antioxidant power (FRAP) method measures the change in absorbance that occurs when the TPTZ (2,4,6-tripyridyl-s-triazine)–Fe^{3+} complex is reduced to the TPTZ–Fe^{2+} form in the presence of antioxidants[21]. The FRAP reagent containing 2.5 mL of TPTZ (10 mM) in HCl (40 mM) plus 2.5 mL of $FeCl_3$ (20 mM) and 25 mL of acetate buffer (0.3 M, pH 3.6) was freshly prepared. *T. capitatus* fractions were dissolved in ethanol at a concentration of 1 mg/mL. An aliquot (0.2 mL) of this solution was mixed with 1.8 mL of FRAP reagent, and the absorption was measured at 595 nm. Ethanol solutions of known Fe(II) concentration in the range of 50–500 µM ($FeSO_4$) were used to obtain the calibration curve. Butylated hydroxytoluene (BHT) was used as positive control.

β-Carotene bleaching test

The antioxidant activity (AA) was determined using the β-carotene bleaching test with some modifications[18]. Concisely, 1 mL of β-carotene solution (0.2 mg/mL in chloroform) was added to 0.02 mL of linoleic acid and 0.2 mL of 100% Tween 20. After evaporation of chloroform and dilution with water, 5 mL of the emulsion was transferred into different test tubes containing 0.2 mL of samples in 70% ethanol at different concentrations (50, 100, 250, 500 and 1000 mg/mL). The tubes were shaken and placed at 45 °C in a water bath for 60 min. The absorbance was measured at 470 nm against a blank consisting of an emulsion without β-carotene. The measurement was carried out at initial time ($t = 0$) and at 30 and 60 minutes. Samples were assayed in triplicate. Propyl gallate was used as positive control. The AA was measured in terms of successful bleaching β-carotene by using the following equation:

$$AA = [1 - (A_0 - A_t/A_0^o - A_t^o)] \times 100$$

where A_0 and A_0^o are the absorbance values measured at the initial incubation time for samples/standard and control, respectively, while A_t and A_t^o are the absorbance values measured in the samples/standard and control, respectively, at $t = 30$ min and $t = 60$ min.

α-Amylase inhibitory activity

The α-amylase inhibition assay was performed using the method previously described[22]. Briefly, a starch solution (0.5% w/v) was obtained by stirring 0.125 g of potato starch in 25 mL of 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65 °C for 15 min. The α-amylase (EC 3.2.1.1) solution was prepared by mixing 0.0253 g of α-amylase in 100 mL of cold distilled water. Samples were dissolved in buffer to give final concentrations ranging from 12.50 µg/mL to 1 mg/mL. The colorimetric reagent was prepared mixing a sodium potassium tartrate solution (12.0 g of sodium potassium tartrate, tetrahydrate in 8.0 mL of 2 M NaOH) and 96 mM 3,5-dinitrosalicylic acid solution. Control and samples were added to starch solution and left to react with α-amylase solution at 25 °C for 5 min. The reaction was measured over 3 min. The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, the product being detectable at 540 nm. Acarbose was used as positive control.

α-Glucosidase inhibitory activity

The α-glucosidase inhibition was measured through a modified Sigma-Aldrich bioassaymethod[22]. A maltose solution (4% w/v) was prepared by dissolving 12 g of maltose in 300 mL of 50 mM sodium acetate buffer. The enzyme solution was prepared by mixing 1 mg of α-glucosidase (10 units/mg) in 10 mL of ice-cold distilled water. Samples were dissolved in the buffer to give a final concentration ranging from 5 µg/mL to 1 mg/mL. The colorimetric reagent DIAN solution was prepared by dissolving one tablet in 25 mL of distilled water, while PGO system-colour reagent solution was prepared fresh by dissolving one capsule in 100 mL of ice-cold distilled water. In the first step both control and samples were added to maltose solution and left to equilibrate at 37 °C. The reaction was started by adding α-glucosidase solution, and tubes were left to incubate at 37 °C for 30 min. After this time a perchloric acid solution (4.2% w/v) was added to stop reaction. In the second step the generation of glucose was quantified by the reduction of DIAN. The supernatant of the tube of step I was mixed with DIAN and PGO and was left to incubate at 37 °C for 30 min. The absorbance of DIAN was measured at 500 nm. Acarbose was used as positive control.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The inhibitory concentration 50% (IC_{50}) was calculated from the Prism dose–response curve (GraphPad Software, San Diego, CA, USA) obtained by plotting the percentage of inhibition versus concentrations. Differences within and between groups were

Table 1. Major nonpolar components tentatively identified in *T. capitatus* leaves—*n*-hexane and methanol fractions.

Compound	*I**	Abundance† *n*-Hexane fraction (HF)	Abundance† Methanol fraction (MF)	Identification‡
α-Pinene	936	0.9 ± 0.01	0.5 ± 0.03	A
p-Cymene	1025	0.6 ± 0.03	2.1 ± 0.01	B
γ-Terpenene	1059	1.8 ± 0.07	4.5 ± 0.01	A
Terpinen-4-ol	1178	1.3 ± 0.02	0.4 ± 0.01	B
Thymol	1290	0.8 ± 0.03	1.6 ± 0.05	A
Carvacrol	1298	35.7 ± 1.9	63.8 ± 2.6	A
β-Caryophyllene	1415	0.7 ± 0.05	2.9 ± 0.12	A
α-Humulene	1447	tr	0.5 ± 0.03	A
γ-Cadinene	1513	0.3 ± 0.03	0.5 ± 0.01	B
δ-Cadinene	1522	tr	tr	B
Caryophyllene oxide	1592	2.8 ± 0.03	0.3 ± 0.03	B
Tetradecanal	1614	0.6 ± 0.05	—	B
Heptadecane	1700	1.4 ± 0.01	0.6 ± 0.04	B
Pentadecanal	1707	1.6 ± 0.01	0.1 ± 0.01	B
Hexadecanal	1811	1.7 ± 0.03	0.8 ± 0.03	B
Neophytadiene	1830	0.5 ± 0.03	2.2 ± 0.01	B
Methyl palmitate	1934	10.3 ± 0.03	1.1 ± 0.01	A
Palmitic acid	1969	4.2 ± 0.22	8.1 ± 0.27	A

*I, Retention index on MS HP-5 nonpolar column.
†Abundance calculated as % peak area mean values, mean ± standard deviation ($n = 3$).
‡The reliability of the identification proposal is indicated by the following: A, mass spectrum and retention index agreed with standards; B, mass spectrum and retention index agreed with database or literature; tr, <0.1%. —, not detected.

evaluated by one-way analysis of variance test (ANOVA) followed by a multicomparison Dunnett's test compared with the positive controls.

Results

The composition of MF and HF obtained from the leaves of *T. capitatus* was investigated by GC and gas chromatography/mass spectrometry (GC-MS) analyses. Data are reported in Table 1. The presence of thymol and carvacrol in *T. capitatus* fractions was preventively evaluated by TLC. The main compound of both *T. capitatus* fractions was carvacrol (63.8 and 35.7% for MF and HF, respectively). The methanol fraction was characterized also by the presence of palmitic acid (8.1%), γ-terpenene (4.5%), β-caryophyllene (2.9%), neophytadiene (2.2%) and *p*-cymene (2.1%) as abundant compounds. Instead, methyl palmitate (10.3%) and caryophyllene oxide (1.8%) are other constituents identified in the HF with good percentage.

The antibacterial activity of *T. capitatus* fractions has been tested on standard strains and on strains isolated from patients with infections of the respiratory tract by evaluating MIC values. The MF was more active than the HF. The MF has been shown to be particularly effective on *Str. pneumoniae* (MIC values of 62.5–125 μg/mL) and *M. catarrhalis* (MIC value of 62.5 μg/mL). Generally, this fraction demonstrated greater antibacterial activity compared to HF with lower MIC values for all strains of all five tested species (MIC value >1000 μg/mL). The antibacterial activity of MF was high also on *Sta. aureus* (MIC of 250 μg/mL) and *H. influenzae* (MIC of 250 μg/mL), suggesting that procedures of extraction allowed obtaining fractions from the leaves of thyme enriched in compounds with the greatest antibacterial property. It has to be also evidenced that the activity of both fractions was independent by the antibiotic resistance of the strains tested.

Table 2. MIC values of *T. capitatus* fractions against pathogenic bacteria.

Strains		MIC (μg/mL) MF	HF	A	B
Gram-positive bacteria					
S. aureus	ATCC 29213	250	>1000	>1000	>1000
S. aureus	MetR	250	>1000	>1000	>1000
S. aureus	MetR	250	>1000	>1000	>1000
Str. pneumoniae	ATCC 49619	62.5	>1000	>1000	>1000
Str. pneumoniae	PenR	62.5	>1000	>1000	>1000
Str. pneumoniae	PenR	125	>1000	>1000	>1000
Str. pneumoniae	EryR	62.5	>1000	>1000	>1000
Str. pneumoniae	EryR	62.5	>1000	>1000	>1000
Str. pneumoniae	PenR EryR	125	>1000	>1000	>1000
Str. pneumoniae	PenR EryR	125	>1000	>1000	>1000
Str. pyogenes	EryS	125	>1000	>1000	>1000
Str. pyogenes	EryR	125	>1000	>1000	>1000
Str. pyogenes	EryR	250	>1000	>1000	>1000
Gram-negative bacteria					
H. influenzae	ATCC 49247	250	>1000	>1000	>1000
H. influenzae	AmpR	250	>1000	>1000	>1000
H. influenzae	AmpR	250	>1000	>1000	>1000
M. catarrhalis	ATCC 25238	62.5	>1000	>1000	>1000

MF, methanol fraction; HF, *n*-hexane fraction; A, methanol; B, *n*-hexane; MetR, methicillin-resistant; PenR, penicillin-resistant; EryR, erythromycin-resistant; EryS, erythromycin-susceptible; AmpR, ampicillin-resistant.

A preliminary evaluation of the antibacterial activity of the individual solvents (methanol and *n*-hexane) was determined by microdilution method according to CLSI[18], and then they were used as negative controls. Confirming the fact that the antibacterial activity of MF and HF fractions was not influenced by solvents, the MIC value of them was always >1000 μg/mL. Results are reported in Table 2.

The AA of *T. capitatus* was evaluated by different *in vitro* systems. A concentration–response relationship was observed for all tested samples. Data are reported in Table 3. The HF showed the highest radical scavenging activity with IC_{50} values of 38.2 and 4.0 μg/mL in DPPH and ABTS tests, respectively. Instead, in the β-carotene–linoleic acid test system *T. capitatus*, MF exhibited the highest bioactivity with IC_{50} values of 0.7 and 1.9 μg/mL after 30 and 60 min of incubation, respectively. Of particular interest is the result obtained after 30 min of incubation if compared with the positive control (IC_{50} value of 1.0 μg/mL). Using the FRAP assay, the results were 27.7 and 32.7 μM Fe(II)/g for MF and HF, respectively.

α-Amylase and α-glucosidase inhibitors are currently used to reduce glucose postprandial plasma level in diabetes type 2 and in case of obesity. In our continuous studies on medicinal plants and edible plants and/or isolated active principles with potential hypoglycaemic effects, herein we have investigated for the first time the *T. capitatus* fractions for their inhibitory activities of carbohydrate-hydrolyzing enzymes[23,24]. A concentration–response relationship was observed. IC_{50} values are reported in Table 4. Both *n*-hexane and methanol fractions are able to inhibit selectively α-amylase with IC_{50} values of 422.5 and 565.9 mg/mL, respectively.

Discussion

The aim of this study is to investigate the MF and HF of *T. capitatus* leaves for their potential antibacterial, antioxidant and hypoglycaemic effects in relation to their main constituents.

The perusal analysis of the literature revealed the presence of several previous studies mainly on the essential oil of *T. capitatus*.

Table 3. The antioxidant properties of *T. capitatus* leaves' methanol and *n*-hexane fractions.

T. capitatus	β-Carotene bleaching test (IC_{50} μg/mL)		DPPH assay (IC_{50} μg/mL)	ABTS assay (IC_{50} μg/mL)	FRAP assay (μmol/L Fe(II)/g)
	30 min	60 min			
Methanol fraction	0.7 ± 0.03*	1.9 ± 0.6*	44.5 ± 1.9†	8.7 ± 0.2†	27.7 ± 2.6†
n-Hexane fraction	27.9 ± 0.9†	42.9 ± 2.3†	38.2 ± 1.2†	4.0 ± 0.8†	32.7 ± 3.5†
Ascorbic acid			5.0 ± 0.8	1.7 ± 0.03	—
BHT			—	—	63.2 ± 4.5
Propyl gallate	1.0 ± 0.04	1.0 ± 0.04			

Data are expressed as means ± SD (standard deviation; $n = 3$). Ascorbic acid, BHT and propyl gallate were used as positive control. One-way ANOVA, ***$p < 0.0001$. Dunnett's multiple comparison test: DPPH test, †$p < 0.01$ (MeOH and *n*-hexane versus ascorbic acid); ABTS test, †$p < 0.01$ (MeOH and *n*-hexane versus ascorbic acid); FRAP test, †$p < 0.01$ (MeOH and *n*-hexane versus BHT); β-carotene bleaching test 30-min incubation, †$p < 0.01$ (*n*-hexane versus propyl gallate), *$p > 0.05$ (MeOH versus propyl gallate); β-Carotene bleaching test 60-min incubation, *$p < 0.01$ (*n*-hexane versus propyl gallate), †$p > 0.05$ (MeOH versus propyl gallate).

Table 4. *T. capitatus* hypoglycaemic activity [IC_{50} (μg/mL)].

T. capitatus	α-Amylase	α-Glucosidase
Methanol fraction	565.9 ± 2.5**	> 1000
n-Hexane fraction	422.5 ± 2.0**	> 1000
Acarbose	50.0 ± 0.9	35.5 ± 1.2

Data are expressed as means ± SD ($n = 3$). Differences within and between groups were evaluated by one-way analysis of variance (ANOVA) test completed with a multicomparison Dunnett's test. **$p < 0.01$ compared with the positive control (acarbose).

Achour et al.[25] demonstrated the presence of carvacrol as main component, followed by *p*-cymene, γ-terpinene, linalyl acetate, 1,8-cineole, β-myrcene, terpinen-4-ol and α-terpinene. Yvon et al.[9] identified 11 compounds in *T. capitatus* oil. The main constituent is thymol, followed by *p*-cymene and γ-terpinene. Carvacrol is present only with a percentage of 0.09%. Previously, carvacrol, *p*-cymene and γ-terpinene were known as the most abundant compounds[26]. The essential oil of *T. capitatus* collected in Egypt showed as main compounds carvacrol and thymol, followed by borneol, sabinene and α-pinene[27]. Recently, Condurso et al.[28] identified carvacrol as major constituent of the oil, followed by γ-terpenene, *p*-cymene, β-caryophyllene, 1-terpinen-4-ol and α-terpinene.

Since two chemotypes, namely, carvacrol and thymol types, have been recognized in *T. capitatus*, on the basis of previously reported data and our results, we can confirm that analysed *T. capitatus* was a carvacrol chemotype.

Taking into account that *T. capitatus* fractions are characterized by the presence of carvacrol as dominant constituent, we can relate the antibacterial properties to the high content of this oxygenated monoterpene. In a previous study, carvacrol and thymol demonstrated the most potent antimicrobial activity against *Escherichia coli, Sta. aureus, Str. epidermidis, Enterococcus faecalis, Yersinia enterocolitica, Candida albicans, Bacillus cereus, Listeria monocytogenes, Salmonella typhimurium* and *Saccharomyces cerevisiae*, with the exception of *Pseudomonas aeruginosa*[29]. Other terpenes were tested for their potential antimicrobial activity. α-Terpineol demonstrated to possess activity against two gram-negative bacteria such as *E. coli* and *S. typhimurium*, while α-pinene, *p*-cymene, γ-terpinene and linalool showed a lower activity against the reference strains at the highest concentration tested. Although carvacrol appears to contribute significantly to the antimicrobial activity of the examined extracts, the possibility that other minor components possess some antimicrobial power still remains. An extract is a complex mixture of compounds, whose biological activity can be the result of a synergism of all constituents or reflect only those of the main molecules present at the highest levels.

The essential oils of *T. satureioides* and *T. pallidus* were tested against a panel of gram-positive and gram-negative bacteria[21]. *B. subtilis* was the most sensitive strain producing an average inhibition zone of 51.7 mm. Furthermore, *P. aeruginosa*, known as a resistant strain, was also sensitive[30].

Recently, the antifungal activity of essential oil and ethanol and hexane extracts of *T. capitatus* against the growth of certain fungi was investigated[31]. Antifungal activity was assessed using the radial growth technique. The highest inhibitory effect on the growth of *Aspergillus niger, A. oryzae, Penicillium digitatum* and *Fusarium solani* was exhibited by the essential oil at concentrations between 0.1 and 0.5 μg/mL.

Thymus species are also known for their antioxidant potential. In particular, the essential oils of *T. leptobotrys* and *T. maroccanus* (carvacrol group) possessed the highest antioxidant activities. *T. satureioides* and *T. pallidus* essential oils showed IC_{50} values of 0.32 and 11.6 mg/mL, respectively, in DPPH assay[32]. The essential oil obtained from the leaves of Tunisian *T. capitatus* showed IC_{50} values of 0.59 and 1.24 mg/L in DPPH and ABTS assay, respectively[9].

Recently, Tabti et al.[31] compared the antioxidant potential of Algerian *T. capitatus* essential oil, ethanol extract and *n*-hexane extract. In the DPPH test, the ethanol extract of *T. capitatus* demonstrated the highest activity, with an IC_{50} value of 31 μg/mL, followed by the hexane extract with an IC_{50} value of 99 μg/mL and the essential oil with an IC_{50} value of 102 μg/mL.

Medicinal plants used by folk medicinal healers are successfully used in many countries to control diabetes and have become the most important sources for seeking safe, specific and effective hypoglycaemic agents[33]. Moreover, many hypoglycaemic components of natural origin include flavonoids, alkaloids, saponins, terpenoids and unsaturated fatty acids[34]. In this context, a recent work demonstrated as the oral administration of the aqueous extract of *T. serpyllum* produced hypoglycaemic effects in alloxan-diabetic-rabbits, protected weight loss and ameliorated diabetic-induced hematological disturbances in rabbits[8]. This is the first study that investigated the hypoglycaemic activity of *T. capitatus* via the inhibition of α-amylase and α-glucosidase, inhibitors that offer an attractive strategy to control postprandial hyperglycaemia for type 2 diabetes management.

Conclusions

In conclusion, *T. capitatus* leaves were investigated for their chemical composition, antibacterial, antioxidant and hypoglycaemic activities. The analysis of results highlights that the methanol fraction showed the most promising antibacterial and antioxidant activities. These results are of a certain interest since demonstrated that the antibacterial activity of *Thymus* sp. is not a prerogative of the essential oil, but it can be also attributed to other type of extracts. Instead, the HF exerted the highest hypoglycaemic activity with a selective action against α-amylase.

These findings demonstrated that both *T. capitatus* leaves' fractions have biological properties that may support the value of carrying out further *in vivo* studies using this species and are a potential source of natural healthy compounds.

Acknowledgements

This work is dedicated to the memory of Prof. Liliana Iauk.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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