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Chemical Composition, Antibacterial and Antioxidant Activities of Thyme Essential Oil (*Thymus vulgaris*)

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Abstract

Herbal medicine from natural resources plays an important role as antibacterial and antioxidant agents. The present investigation was designed to evaluate the antibacterial and antioxidant properties of thyme (*Thymus vulgaris* L.) essential oil (TEO) and/or chitosan (CH) *in vitro*. Results indicated that TEO exhibited high radical scavenging activity (RSA) toward DPPH, ABTS, linoleic acid deterioration and iron chelation activity. TEO exhibited high amount of total phenolic compounds (TPC) related to its terpenes. The TPC of TEO was 177.3 mg GAE g^{-1} demonstrated 149.8 µmol of TE g^{-1} DPPH-RSA and 192.4 µmol of TE g^{-1} ABTS-RSA. The antioxidant capacity of TEO exhibited 68.9% reduction when evaluated by β-carotene bleaching assay. The reducing power activity related to iron chelation was 142.8 µmol of AAE g^{-1}. The TEO exhibited a high content of Thymol (41.04%) as major compound over 14 identified components by GC-MS analysis followed by 1,8-Cineole (14.26%), γ-Terpinene (12.06%), p-Cymene (10.50%) and α-Terpinene (9.22%). TEO exhibited antimicrobial activity *in vitro* and MIC noticed that TEO was efficiently affected pathogens *in vitro*. Indeed, CH exhibited negligible or very low antimicrobial activity. In conclusion, both investigated TEO and TEO-CH_{mix} have strong antibacterial activity against many pathogenic bacteria and need exploitation as an alternative source of natural antibacterial and antioxidant agents for potential applications.

Keywords

Thymus vulgaris, Essential Oil, Antimicrobial Activity, Antioxidant Activity

1. Introduction

Currently, a significant number of pioneer drugs are separated, purified from plants which contained bioactive compounds against a number of different diseases. The World Health Organization (WHO) reported that approximately 80% of the world's population remain depending on a wide range of traditional medicines [1]. Antimicrobial properties of herbs and spices have been recognized and used since ancient times for food preservation and in the traditional medicine. Numerous studies have documented that essential oils played a key-role and presented a great antibacterial effects against a wide range of microbial species (including bacteria, fungi and candida) cited in [2]. The antimicrobial properties of essential oils come from numerous plants have been empirically recognized for centuries, however they are scientifically confirmed only since few years [3]. Bioactive compounds derived from natural resources (such as plants, microbial isolates, algae) have received a great interest due to the pharmacological activities, medicinal properties, low adverse effects and above all economic viability. Essential oils are considered as an antibacterial, antifungal, antiviral, insecticidal and antioxidant bio-agent due to their biologically active compounds, *i.e.* carvacrol, eugenol and thymol [4]. *Thymus vulgaris* is a well-known plant with aromatic characteristics which is frequently used as a spice and herbal since ancient era. Thyme (*Thymus vulgaris*) essential oil (TEO) is enriched source with a wide range of aromatic bioactive components such as thymol and carvacrol, which act considerable role as antioxidative and antimicrobial agents [5]. Owing to the negative clinical impacts and the adverse side-effects of overusing synthesized medicine, extensive studies have currently been conducted on the commercial applications of essential oils and their constituent's (extracted from natural sources) as antimicrobial, antioxidant agents by several researchers. Burt *et al.* [6], reported that TEO contains mainly carvacrol, thymol, *p*-cymene and *γ*-terpinene. These bioactive fractions are not only responsible for the antimicrobial activity but also contained phenolic compounds which are responsible for the high antioxidant capacity of thyme. In addition, Braga *et al.* [7] established that thymol has significant effects in controlling the inflammatory mechanism present in many infections, which are essential for proper wound remedy. Since inflammation causes many complications including wound dehiscence, infection and impaired collagen synthesis, thus anti-inflammatory effects of thymol would be a promising route naturally [8]. The antimicrobial action is normally considered as resulted by disturbing the function of the cytoplasmic membrane, disrupting the active transport of nutrients to the cell membrane, and coagulation of microbial cell contents [9]. Despite significant findings for wound healing by applying a variety of medicinal plants such as *Rubia cordifolia*

Linn, *Ocimum kilimandscharicum*, *Tephrosia purpurea* Linn, *Aloe vera* Linn, and *Napoleona imperialis*, however Thyme gained currently more attention due to its dual or triple actions (antioxidant, antimicrobial and wound healing). On the other hand, chitosan (CH) has been proven to be a nontoxic, biodegradable, bio-functional, biocompatible and has antimicrobial characteristics [10]. The film-forming property of CH has found many claims in tissue of culture and drug delivery, packaging by virtue of its mechanical strength and above all, rather slow biodegradation [11]. CH promotes valuable wound healing properties because of its rapid dermal reformation, accelerated wound regeneration besides its bacteriostatic effects [12]. Wound healing is a complicated process involving various mechanisms, *i.e.* coagulation, matrix synthesis, inflammation and deposition, angiogenesis, epithelization, fibroplasia, contraction and remodeling [13]. There are studies showing that CH has the clinical capability to accelerate wound healing effectively [14]. Therefore, the main objective of this research was to assess antioxidant and antimicrobial efficiency of TEO and/or CH to have a basic information for further application of TEO and CH in wound healing application. In order to achieve this goal, antioxidant and antimicrobial activities of CH, TEO and CH-TEO (mixture 1:1) were assessed *in vitro*. In addition, chemical composition of TEO by GC-MS analysis was determined.

2. Materials and Methods

2.1. Chemicals

DPPH, 1,1-diphenyl-2-picrylhydrazyl; $ABTS^{\bullet+}$, 2.2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-carboxylic acid; GA, gallic acid; TCA, Trichloroacetic acid; AA, ascorbic acid and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigam, Germany, while Tween 80 were obtained from Eugene, Oregen, USA. CH, [poly B-(1,4) N-acetyl-D-glucosamine], low molecular weight with deacetylation degree of 95%, Oxford laboratory reagent, Mumbai, India.

2.2. Essential Oil

Highlypure grade of dried herbs of thyme (*T. vulgaris*) (TEO) was obtained from the Fragrance and Extraction Factory, Sugar Industrial Integrated Company (SIIC), Cairo, Egypt using the hydro-distillation closed system.

2.3. Chitosan Preparation

A 2.0% (w/v) chitosan solution was prepared by dissolving CH in 0.1% acetic acid solution. It was stirred till complete dissolving, then CH solution was placed for 24 h in a heater at 37˚C under vacuum to favor acetic acid evaporation.

2.4. Bacterial Strains

Bacterial strains such as (*Bacillus cereus, Escherichia coli, E. coli* O16*, E. coli*

O26: H11, *E. coli* O103: H2, *E. coli* O121, *E. coli* O157: H7, *Listeria monocytogenes*, *Salmonella typhi*, *S. typhmurium*, *Staphylococcus aureus* and *Yersinia Spp.*) were obtained from microbiological laboratory of Agricultural botany department, Faculty of Agriculture, Benha Univ., Egypt, Institute for Fermentation (Institut für Gärungsgewerbe, Berlin, Germany), and Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams Univ., Cairo, Egypt.

2.5. Determination of Total Phenolic Content (TPC)

The total phenolic content of TEO was determined using the reagent of Folin-Ciocalteu according to modified method by Bettaieb *et al.* [15]. A prepared standard curve of Gallic acid (GA) in range of 50 - 500 mg·ml^{-1} was used to compare the measurements (R^2 = 0.99), the total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of TEO (mg of GAE g^{-1}).

2.6. Antioxidant Activity

2.6.1. DPPH Radical Scavenging Assay

Radical scavenging activity of TEO was assayed according to modified method by Lu *et al.* [16]. Trolox calibration curve was plotted as a function of percentage of DPPH radical scavenging activity. The antiradical activity was presented as micromoles of trolox equivalents (TE) per gram of TEO (μmol TE g^{-1}).

2.6.2. ABTS Radical Cation Scavenging Activity

Radical scavenging activity of TEO against ABTS radical cation was measured using the modified method of Lu *et al.* [16]. Results were presented as micromoles of trolox equivalents (TE) per gram of TEO (μmol of TE g^{-1}).

2.6.3. *β*-Carotene-Linoleic Acid Bleaching Assay

A modified spectrophotometric method is described by Koleva *et al.* [17] modified by Barakat [18] was employed. The antioxidant activity (%) of TEO was evaluated in terms of the bleaching of the *β*-carotene relating to BHA. The results were expressed as BHA-related percentage.

2.6.4. Chelating Effect on Ferrous Ions

Ferrous ion chelation activity of TEO was assessed as described by Zhao *et al.* [19]. The inhibition percentage of ferrozine-Fe^{2+} complex formation as metal chelation activity was calculated and expressed as (mg·mL^{-1}) when EDTA was used as a positive control.

2.6.5. Reducing Power Assay

Determination of reducing power was carried out as described by Oktay *et al.* [20]. The measurements were compared to prepared ascorbic acid (AA) standard curve, and final results were presented as micromoles of ascorbic acid equivalents (AAE) per gram of TEO (μmol of AAE g^{-1}).

2.7. Gas Chromatography Mass Spectrometry (GC-MS)

The chemical composition of the essential oil was analyzed using GC-MS technique according to Cosentino *et al.* [21]. The essential oils were chromatographed using a Shimadzu gas chromatograph QP2010-GC-MS with auto-sampler under suitable conditions. The components of TEO were identified by comparing their relative retention times and mass spectra with identified and known compounds stored in the internal library.

2.8. Antibacterial Activity

2.8.1. Inhibitory Effect by Agar Disk-Diffusion Method

The determination of the inhibitory effect of pure TEO, mixed pure TEO with 2% CH (TEO-CH_{mix}) and 2% CH solution against bacterial strains was carried out by the agar disk-diffusion method [22] Similarly, the antimicrobial activity of 16 popular antibiotics have been used and compared with TEO. For examine TEO efficiency, the results were calculated basically from the obtained inhibition zone results of antibiotics and TEO.

2.8.2. Minimum Inhibitory Concentration of TEO

The microdilution broth susceptibility assay was used according to Lambert *et al.* [23] with modification. Appropriate interval concentration from TEO, TEO-CH_{mix} and CH in Mueller-Hinton Broth (MHB) was prepared. A 96-well plates were settled by dispensing into each well, 195 µl from each previously prepared mixture and 5 µl of the inoculant of each strain (10^6 mL^{-1}). The inoculums of microorganisms were prepared using 24 h cultures and suspensions were adjusted to 4 McFarland standard turbidity. Final volume in each well was 200 µl. A positive control (containing inoculum but not TEO, TEO-CH_{mix} or CH) and negative control (containing TEO, TEO-CH_{mix} or but no inoculums) were included on each microplate. The microplates were incubated at 37˚C for 48 h. The experiment was carried out in triplicate and three replicates of each microassay were done. The lowest concentration of the compounds which inhibited the growth of microorganisms is defined as MIC.

2.9. Statistical Analysis

SPSS program regarding to the experimental design under significance level of 0.05 was used for statistical analysis according to Steel *et al.* [24]. Pearson's correlation analysis was done and obtained correlation results were compared to critical values of Pearson's *r* table under levels of significance with one-tailed test as calculated by Barakat and Rohn [25].

3. Results and Discussion

3.1. Total Phenolic Content and Antioxidant Activity of TEO

The amounts of total phenolic content (TPC) in the TEO had been determined spectrometrically and calculated as mg GAE g^{-1} as well as the antioxidant activi-

ties of TEO by the DPPH radical scavenging, ABTS, the β-carotene-linoleic acid bleaching, chelating ability and the reducing power were carried out. As seen in Table 1, the TPC amount of TEO reached to 177 mg GAE g^{-1} of TEO. Obtained results exhibited that DPPH radical cation scavenging activity (DPPH-RSA) of TEO was 150 µmol of TE g^{-1}. Moreover ABTS-RSA was used to determine the evolution of antioxidant activity of TEO, and results are presented in Table 1. Compared with DPPH-RSA, the ABTS-RSA of TEO samples was affected similarly to present 192 µmol of TE g^{-1}. Furthermore, the relative antioxidative activity (RAAs) of TEO is given in Table 1. The inhibition values of linoleic acid radicals were estimated as 69% compared to BHA. A positive relationship between the DPPH scavenging ability, ABTS and β-carotene bleaching extent was confirmed. Evaluation of the metal chelating power revealed 39 mg·g^{-1} which seems to be capable of interfering with Fe^{2+}-ferrozine complex formation, suggesting its ability to capture ferrous ions before ferrozine. Data in Table 1, illustrated the evolution of reducing power of TEO which was 143 µmol of AAE g^{-1}. It is worth mentioning that, according to these results, there is a positive relationship between the TPC and antioxidant activities. Phenolic compounds as biologically active components break chain reaction of lipid oxidation at first initiation step by donating hydrogen to free radicals. This high activity of phenolic compounds to scavenge radicals may be explained by their phenolic-hydroxyl groups [26]. The high chelating power of TEO could prevent transition-metal ions exuding desirable reduction in lipid peroxidation. Generally, a positive correlation between TPC and antioxidant capacity is reported. Thus, this high performance of the TEO is related to their phenolic composition. Recently, it has been shown that the antioxidant activity of extracts is roughly connected to their phenolic composition and strongly depends upon their phenolic structures. These phenolic acids have been reported as an efficient antioxidant compound, scavenging reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and hydroxyl radical [27]. Moreover, Andjelkovic *et al.* [28] confirmed the capacity of several phenolic acids to form complex with iron ion and attended the oxidation. Innovatively, combines antioxidant properties with antimicrobial activities showed wound healing activity as encouraged by Altiok *et al.* [4].

Table 1. Total phenolic content and potential antioxidant activities of thyme EO (mean ± SE).

Item	Thyme EO
TPC (mg GAE g^{-1})	177.3 ± 1.9
DPPH (µmol of TE g^{-1})	149.8 ± 6.7
ABTS (µmol of TE g^{-1})	192.4 ± 3.9
B-carotene bleaching* (RAA)%	68.9 ± 3.2
Chelating ability (mg·g^{-1})	38.5 ± 1.7
Reducing power (µmol of AAE g^{-1})	142.8 ± 6.1

*: relatively calculated based on BHA activity as 100%.

3.2. Pearson's Correlation Coefficients of TPC and Different Antioxidant Activities of TEO

Pearson's correlation coefficients were calculated to determine the conceivable correlation between TPC and their different antioxidant capacities (Table 2). Very highly significant correlations have been observed mostly between TPC and potential antioxidant activities of TEO and among others. Surely, this varied significantly correlation demonstrated the efficiency of TEO to struggle different synthetic radicals which assayed by $DPPH^{\bullet}$, $ABTS^{\bullet+}$, β-carotene bleaching, chelating ability and reducing power assays. This very high significant correlation confirms the potential antioxidant capacity of TEO to combat varied oxidation systems. In the same context, similar finding had been recorded previously [25] [29].

3.3. Composition of *T. vulgaris* Essential Oil Determined by GC-MS

Fourteen separated components were identified by GC-MS in *T. vulgaris* EO considered as 95.77% of TEO compounds, data were demonstrated in Table 3. The major compound of TEO was Thymol (41.04%) whereas, 1,8-Cineole (14.26%), γ-Terpinene (12.06%), p-Cymene(10.50%), α-Terpinene (9.22%), Linalool (2.80%) and Carvacrol (2.77%) were observed in valuable amounts. Essential oils are rich in phenolic compounds such as 1,8-Cineole, α-Pinene, β-Pinene, α-Terpineol and Camphor are widely reported to possessing high levels of antioxidant and antimicrobial activities [30] [31]. In the present study, thymol was the major volatile constituent of TEO which is a phenolic component that has antioxidant and antimicrobial capacities [32] [33]. Over many recent literatures, the variations in chemical composition of essential oils were depending on climatic, seasonal, and geographic conditions [34]. Our results are in agreement with Sacchetti *et al.* [35] who's identified more components in *T. vulgaris* include major presented components of tested *T. vulgaris* with high antioxidant and antiradical capacities. The Thymol, 1,8-Cineole and γ-Terpinene were mostly identified compounds in many recent studies [32] [36].

Table 2. Pearson's correlation coefficients of TPC and different antioxidant activities of TEO.

Item	TPC	DPPH	ABTS	*B*-carotene bleaching	Chelating ability	Reducing power
TPC	1.00	0.78**	0.58*	0.87***	0.72**	0.65*
$DPPH^{\bullet}$		1.00	0.88***	086***	0.81***	0.78**
$ABTS^{\bullet+}$			1.00	0.79***	0.95***	0.89**
B-carotene bleaching				1.00	0.84***	0.93***
Chelating ability					1.00	0.86***
Reducing power						1.00

Asterisks (*, ** and ***) represent a significant difference at ($p < 0.05$, $p < 0.01$ and $p < 0.005$), respectively.

Table 3. Major components of *T. vulgaris* essential oil determined by GC-MS.

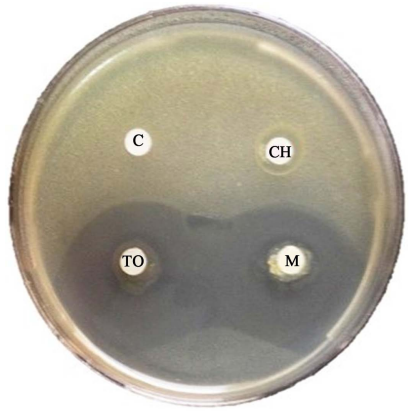
N°	Compound[a]	R_t	%	K.I.	Method of identification
1	Myrcene	13.61	0.04	990	MS, RI, Lit.
2	*α*-Terpinene	14.36	9.22	1020	MS, RI, Lit.
3	*p*-Cymene	14.46	10.50	1026	MS, RI, Lit.
4	1,8-Cineole	15.18	14.26	1035	RI, Lit.
5	Thymol	16.40	41.04	1065	MS, RI, Lit.
6	Terpinolene	16.49	0.25	1088	RI
7	Linalool	17.59	2.80	1097	MS, RI, Lit.
8	trans-Thujone	18.62	0.22	1109	MS, RI
9	Terpin-4-ol	25.17	0.65	1181	MS, RI, Lit.
10	*α*-Terpineol	25.42	1.10	1193	RI, Lit.
11	cis-Carveol	25.82	0.43	1229	MS, RI
12	*γ*-Terpinene	26.23	12.06	1237	MS, RI, Lit.
13	Carvacrol	29.01	2.77	1301	MS, RI, Lit.
14	Caryophyllene	33.56	0.43	1403	MS, RI, Lit.
				95.77	
	Unidentifiable compounds			4.33	
	Total			100	

[a]:Tentatively identified compounds; R_t: Retention time in minutes; RI: Retention index; MS: Mass spectrum; Lit.: Literature review.

3.4. Antimicrobial Activity of TEO *in Vitro*

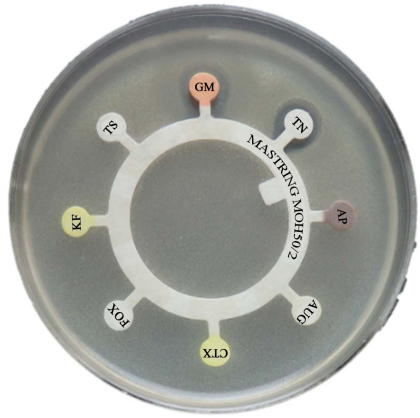
Indeed, there has been significant attention in essential oils with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms [37], as well as for assisting wound to be healed rapidly [38]. The antimicrobial activity of CH, TEO and TEO-CH_{mix} against some pathogenic strains to be used latterly as wound healing promotors has been investigated. The obtained results in **Table 4** and **Figure 1**, illustrated that the highest effective agent against tested pathogenic strains was TEO which showed inhibition zone ranged from 25 to 38 mm with relative MIC ranged from 40 to 270 $mg{\cdot}L^{-1}$. The highest inhibitory activity was recorded for TEO followed by TEO-CH_{mix} and CH. The highest sensitive bacterial strain was *L. monocytogenes* which showed the largest inhibition zones as 38, 28 and 12 mm and lowest MIC (40, 90 and 11,000 $mg{\cdot}L^{-1}$) for TEO, TEO-CH_{mix} and CH, respectively. On contrary, the lowest inhibitory activity was against *S. Typhimurium* which showed the narrowest inhibition zones as 25, 18 and 7 mm and the highest MIC (270, 520 and 95000 $mg{\cdot}L^{-1}$) for TEO, TEO-CH_{mix} and CH, respectively (**Table 4**). The inhibition zones of the essential oil for each assay on test bacteria showed a significant correlation with MIC values. The results are in agreement with [3] [39].

TEO exhibited better antibacterial activity when compared with the commercial antibiotics (**Table 5**). The results show that TEO have very good antibacterial



(a)

(b



(c)

Figure 1. Antimicrobial activity of 2% chitosan solution (CH), thyme oil mixed with 2% chitosan solution (1:1, v:v) (M) and pure (TEO) on *E. coli* O157: H7 as exemplary shown and compared with (C), as control disk contains sterile 0.1% acetic acid solution, **Figure 1(a)**, in comparing with different antibiotics **Figure 1(b)** and **Figure 1(c)**. Antibiotics abbreviations indicated as, AK: Amikacin 30 μg, ATM: Aztreonam 30 μg, C: Chloramphenicol 30 μg, CAZ: Ceftazidime 30 μg, IMI: Imipenem 10 μg, CIP: Ciproflaxacin 1 μg, PRL: Piperacillin 100 μg and T: Tetracycline 30 μg (**Figure 1(b)**). AP: Ampicillin 10 μg, AUG: Augmentin 30 μg, CTX: Cefotaxime 30 μg, FOX: Cefoxitin 30 μg, KF: Cephalothin 30 μg, TS: Cotrimoxazole 25 μg, GM: Gentamicin 10 μg and TN: Tobramycin 10 μg (**Figure 1(c)**).

Table 4. Inhibitory effect of with CH, TEO and TEO-CH_{mix} by agar disk-diffusion assay and their MIC against some pathogenic bacterial strains *in vitro*.

Organism	Gram	Inhibition zone diameter [mm]*			MIC [mg·L^{-1}]		
		CH	TEO	TEO-CH_{mix}	CH	TEO	TEO-CH_{mix}
B. ceruse	+	8.00 ± 1.0^{a}	35.00 ± 1.0^{c}	31.00 ± 1.2^{b}	8500 ± 50^{c}	250 ± 31^{a}	470 ± 42^{b}
E. coli	−	8.00 ± 1.0^{a}	25.00 ± 1.0^{b}	24.00 ± 1.0^{b}	7200 ± 35^{c}	110 ± 17^{a}	190 ± 22^{b}
E. coli O16	−	12.33 ± 0.6^{a}	31.33 ± 0.6^{c}	26.33 ± 0.6^{b}	6800 ± 48^{c}	70 ± 09^{a}	150 ± 23^{b}
E. coli O26	−	9.33 ± 0.6^{a}	26.33 ± 0.6^{c}	21.33 ± 0.6^{b}	8400 ± 25^{c}	80 ± 12^{a}	170 ± 19^{b}
E. coli O103	−	9.67 ± 1.2^{a}	26.67 ± 1.2^{c}	21.67 ± 1.2^{b}	8700 ± 85^{c}	100 ± 25^{a}	190 ± 21^{b}
E. coli O121	−	10.00 ± 1.0^{a}	27.00 ± 1.0^{c}	22.00 ± 1.0^{b}	6900 ± 55^{c}	90 ± 15^{a}	180 ± 35^{b}
E. coli O157: H7	−	9.67 ± 1.53^{a}	25.67 ± 1.5^{b}	24.67 ± 1.5^{b}	7900 ± 49^{c}	120 ± 22^{a}	210 ± 21^{b}
L. monocytogenes	+	12.00 ± 1.0^{a}	38.00 ± 1.0^{c}	28.00 ± 1.0^{b}	11000 ± 99^{c}	40 ± 15^{a}	90 ± 17^{b}
S. Typhimurium	−	6.67 ± 0.6^{a}	25.67 ± 1.5^{c}	17.67 ± 1.53^{b}	95000 ± 87^{c}	270 ± 12^{a}	520 ± 18^{b}
S. typhi	−	9.00 ± 1.0^{a}	25.33 ± 1.5^{c}	22.67 ± 0.6^{b}	5000 ± 85^{c}	230 ± 13^{a}	450 ± 12^{b}
Staph. aureus	+	8.67 ± 1.0^{a}	30.67 ± 1.2^{b}	29.67 ± 1.2^{b}	7500 ± 79^{c}	80 ± 11^{a}	190 ± 17^{b}
Yersinia spp.	−	11.00 ± 1.0^{a}	28.00 ± 1.0^{c}	23.00 ± 1.0^{b}	12000 ± 13^{c}	90 ± 15^{a}	160 ± 12^{b}

*: Results includes paper disc [6 mm].

Table 5. Antibiotics equivalent (μg) of 1 μl pure TEO tested against some pathogenic bacterial strains *in vitro,* data were calculated basically from disk-diffusion assay results.

Organism	Antibiotics							
	GM	TN	T	AK	C	IMI	CIP	PRL
B. ceruse	0.97 ± 0.11^{cd}	1.23 ± 0.17^{c}	4.38 ± 0.32^{b}	4.12 ± 0.14^{b}	4.12 ± 0.32^{b}	0.86 ± 0.07^{d}	0.16 ± 0.01^{e}	11.11 ± 1.05^{a}
E. coli	0.79 ± 0.21^{c}	1.52 ± 0.24^{bc}	2.50 ± 0.52^{b}	2.0 ± 0.42^{b}	2.94 ± 0.24^{b}	0.69 ± 0.07^{c}	0.12 ± 0.01^{e}	8.33 ± 0.57^{a}
E. coli O16	1.16 ± 0.24^{d}	1.90 ± 0.18^{c}	3.48 ± 0.24^{b}	4.18 ± 0.34^{b}	3.48 ± 0.27^{b}	0.67 ± 0.09^{e}	0.12 ± 0.02^{f}	9.49 ± 0.54^{a}
E. coli O26	0.84 ± 0.11^{e}	1.46 ± 0.24^{d}	2.93 ± 0.41^{c}	4.05 ± 0.25^{b}	2.77 ± 0.27^{c}	0.8 ± 0.08^{e}	0.12 ± 0.03^{f}	7.98 ± 0.25^{a}
E. coli O103	1.05 ± 0.09^{e}	1.78 ± 0.24^{d}	2.96 ± 0.27^{c}	4.10 ± 0.27^{b}	2.81 ± 0.17^{c}	0.81 ± 0.5^{f}	0.12 ± 0.02^{g}	8.08 ± 0.24^{a}
E. coli O121	1.29 ± 0.21^{c}	1.50 ± 0.18^{c}	3.18 ± 0.31^{b}	3.86 ± 1.02^{b}	3.18 ± 0.24^{b}	0.64 ± 0.01^{d}	0.11 ± 0.0^{e}	9.00 ± 0.75^{a}
E. coli O157: H7	0.74 ± 0.08^{e}	1.71 ± 0.07^{d}	3.21 ± 0.24^{c}	3.95 ± 0.15^{b}	2.70 ± 0.27^{c}	0.57 ± 0.14^{f}	0.10 ± 0.02^{g}	7.13 ± 0.98^{a}
L. monocytogenes	2.11 ± 0.47^{d}	2.53 ± .61^{d}	4.47 ± 0.85^{c}	6.91 ± 0.35^{b}	4.22 ± 0.29^{c}	0.97 ± 0.08^{e}	0.16 ± 0.01^{f}	11.01 ± 0.48^{a}
S. Typhimurium	1.32 ± 0.24^{c}	1.71 ± .51^{c}	2.85 ± 0.27^{bc}	3.67 ± 0.29^{b}	3.21 ± 0.24^{bc}	0.74 ± 0.12^{d}	0.10 ± 0.01^{e}	7.78 ± 1.09^{a}
S. typhi	1.21 ± 0.24^{c}	1.41 ± 0.42^{c}	2.81 ± 0.21^{b}	3.38 ± 0.24^{b}	2.67 ± 0.17^{b}	0.63 ± 0.11^{d}	0.11 ± 0.01^{e}	8.44 ± 2.40^{a}
Staph. aureus	1.28 ± 0.14^{d}	1.46 ± 0.24^{d}	3.41 ± 0.24^{c}	4.09 ± 0.24^{b}	3.41 ± 0.27^{c}	0.89 ± 0.19^{e}	0.14 ± 0.02^{f}	10.76 ± 2.01^{a}
Yersinia spp.	0.93 ± 0.32^{d}	1.56 ± 0.24^{c}	3.11 ± 0.24^{b}	4.00 ± 0.35^{b}	3.29 ± 0.24^{b}	0.67 ± 0.28^{d}	0.10 ± 0.01^{f}	7.78 ± 1.02^{a}

GM: Gentamicin, TN: Tobramycin, T: Tetracycline, AK: Amikacin, C: Chloramphenicol, IMI: Imipenem, CIP: Ciproflaxacin and PRL: Piperacillin.

activity and can be exploited against *S. aureus* (responsible for bases, sepses and skin infection) and *B. subtilis* (infection in immune compromised patients) as mentioned [40]. TEO can also be used to control *E. coli* (responsible for urinogenital tract infections and diarrhoea). These findings suggest that investigated TEO or TEO+CH have superior antibacterial activity against many human and food pathogenic bacteria and need exploitation as an alternative source of natural antibacterial agents for curing and wound applications [41] [42] [43].

4. Conclusion

Thyme (*T. vulgaris*) essential oil (TEO) showed high amount of TPC with high radical scavenging activity toward DPPH, ABTS and linoleic acid radicals as well as chelating activity toward iron element. The composition of TEO exhibits a high thymol (41.04%) over 14 identified components by GC-MS. The MIC of TEO exhibited antimicrobial activity at low concentrations against tested pathogenic bacteria in the range of 40 - 270 $mg{\cdot}L^{-1}$, *in vitro*. The TEO can be reliably used in commercial applications as antimicrobial and antioxidant agent in individual or in combination with common preservatives for controlling the undesirable organoleptic and microbial deterioration in some food modules or as wound healing curing agent. Interestingly, TEO had strong antibacterial activity against many pathogenic bacteria better than standard antibiotics and need exploitation as an alternative source of natural antibacterial agents for wound applications.

Author Disclosure Statement

The authors declare no conflict of interest.

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