Synergistic Effect of Honey and *Thymus Ciliatus* Against Pathogenic Bacteria

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Abstract: The emergence of pathogenic bacterial strains with resistance to commonly used antibiotics has necessitated a search for novel types of antibacterial agents. The main objective of this study was to investigate the antibacterial activities of the powder of *Thymus ciliatus* and wild carrot honey when used jointly by the determination of MIC (Minimum Inhibitory Concentration) against three pathogenic bacteria, namely *Staphylococcus aureus* OxaR ATCC 43300, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. The results indicate that the powder of thyme and honey are efficient against the tested strains. Honey MIC values were 10%, 14%, 10% (vol/vol), respectively whereas the MIC values of thyme powder were 0.4%, 2%, 9% (W/vol), respectively. When honey and thyme powder are used jointly, we noticed a decrease of the MIC values which is may be due to their synergistic effect. These preliminary results suggest that honey and thyme could be used together to manage superficial wounds and bacterial infections.

Keywords: Honey, *Thymus ciliatus*, antibacterial, synergy.

INTRODUCTION

Currently many questions are rising regarding the security of the chemical compounds used in medicine or in food industry [1]. On the other hand, the extensive use of antibacterial chemical compounds in human medicine and animal farming was conducted to the selection of resistant bacterial strains [2]. The history of aromatic and medicinal plants associate the valuation of the world civilization, the history of people shows that these plants occupy an important place in medicine, perfume composition and culinary preparation [3]. The aromatic plants have traditionally been used in folk medicine as well as to extend the shelf life of foods; showing inhibition against bacteria, fungi and yeasts [4]. Aromatic plants and spices have great importance for humans and despite many of them were substituted by synthetic ones, the demand for natural products is increasing [5]. During the past decade, traditional systems of medicine have gathered global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phyto-medicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs. Few plant species of medicinal herbs have been scientifically evaluated for their possible medical application. *Thymus* (thyme) is one of the most important genera regarding numbers species within the *Lamiaceae* family. This genus is distributed in the Old World and on the coasts of Greenland, from the Macaronesian Region, Northern Africa and the Sinai Peninsula, through the West and East Asia. However, the central area of this genus surrounds the Mediterranean Sea [6-8]. The plants of *Thymus* genus are among the most popular plants throughout the world, commonly used as herbal teas, flavoring agents (condiment and spice), aromatic, and medicinal plants [9]. *Thymus* species are known to be used for traditional medicine for the treatment of various illnesses and have been found to possess significant pharmacologic activities [8, 10, 11]. These species have also been used as carminative, diuretic, urinary disinfectant and vermifuge [12]. The antioxidant and antimicrobial activity of the members of the genus *Thymus* have been determined [13-16]. Honey has been used for medicinal purposes since ancient times in the treatment of wounds and burns. After having played an important part in the traditional medicine for centuries, honey was subjected to laboratory and clinical investigations during the past few decades [17] and found to have important healing and anti-inflammatory properties [18]. Recently, the use of honey has re-emerged, mainly due to clinical observations of its antimicrobial effect and accelerated wound healing [18, 19]. Various studies attribute antibacterial, antifungal, anti-inflammatory, antiproliferative

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Table 1. MIC Value of Honey and *Thymus ciliatus*

Bacterial Strains	MIC Values of Honey and *Thymus Ciliatus*	
	Honey	*Thymus Ciliatus*
S. aureus ATCC 43300	10%	0.4%
E. coli ATCC 29523	14%	2%
P. aeruginosa ATCC 27853	10%	9%

Table 2. Value of MSIC against Cagainst *S. aureus*

Honey (%)	9	8	7	6	5	4	3	2	1
Powder (%)	0,1	0,1	0,1	0,15	0.15	0 ,2	0,2	0,2	0,2

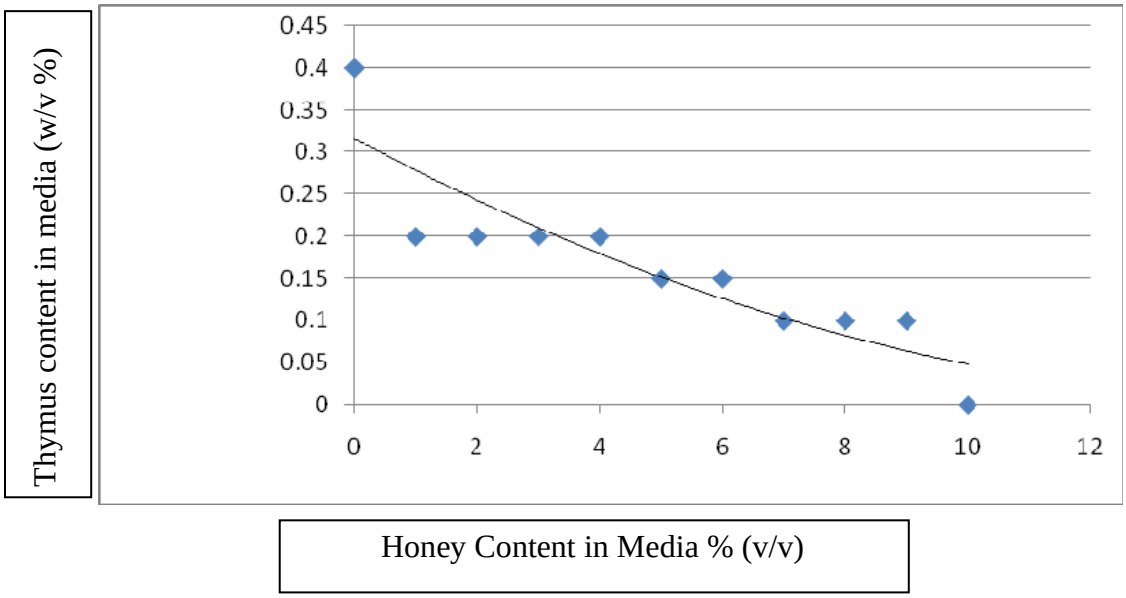

Fig. (1). Isobologram representing over additive effect of *Thymus* and honey against *Staphylococcus aureus oxaR.*

Table 3. Value of MSIC against *Pseudomonas aeruginosa*

Honey (%)	9	8	7	6	5	4	3	2	1
Powder (%)	2	2	2	4	4	5	6	7	7

and anticancer potentiating properties to honey [20]. The objective of this study is to evaluate the synergetic effect of the powder of *Thymus ciliatus* and wild carrot honey in terms of antibacterial activity against three bacterial strains.

MATERIALS AND METHODS

Vegetal Material

The aerial part of *Thymus ciliatus* was dried at 35°C in the dark then grinded, sieved and autoclaved.

HONEY

Monofloral honey sample (*Daucus carota* L) was kindly provided by a local beekeeper during the year 2010.

BACTERIAL STRAINS AND INOCULUMS STANDARDIZATION

S. aureus OxaR 43300, *E. coli* ATCC 25922 *and P. aeruginosa* ATCC 27853 were kindly provided by the university hospital Mustapha Pasha of Algiers (Algeria). Prior to the experiment the strains were maintained by subculture

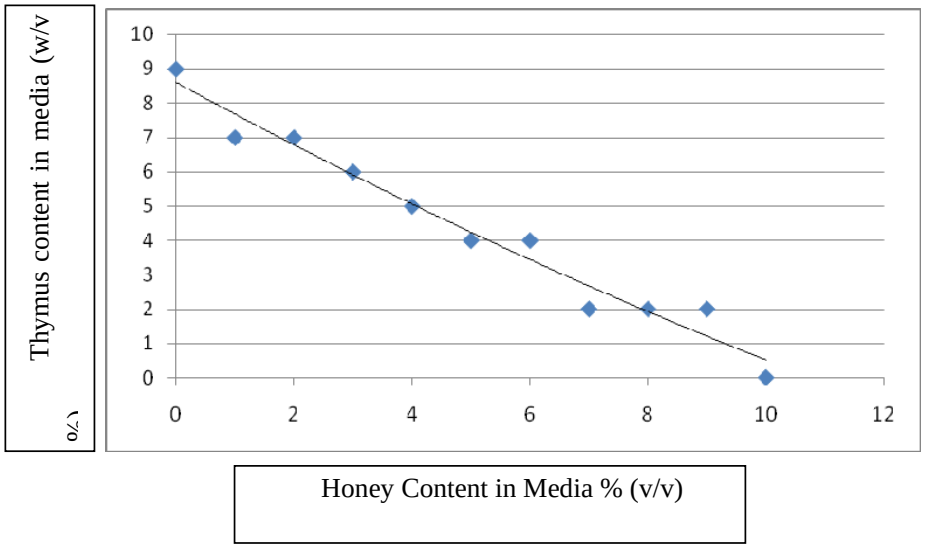

Fig. (2). Isobologram representing additive effect of *Thymus* and honey against *Pseudomonas aeruginosa.*

Table 4. Value of MSIC of *E. coli*

Honey (%)	13	12	11	10	9	8	7	6	5	4	3	2	1
Powder (%)	0,15	0,15	0,15	0,15	0,7	0,8	1,3	1,3	1,4	1,4	1,5	1,7	1,8

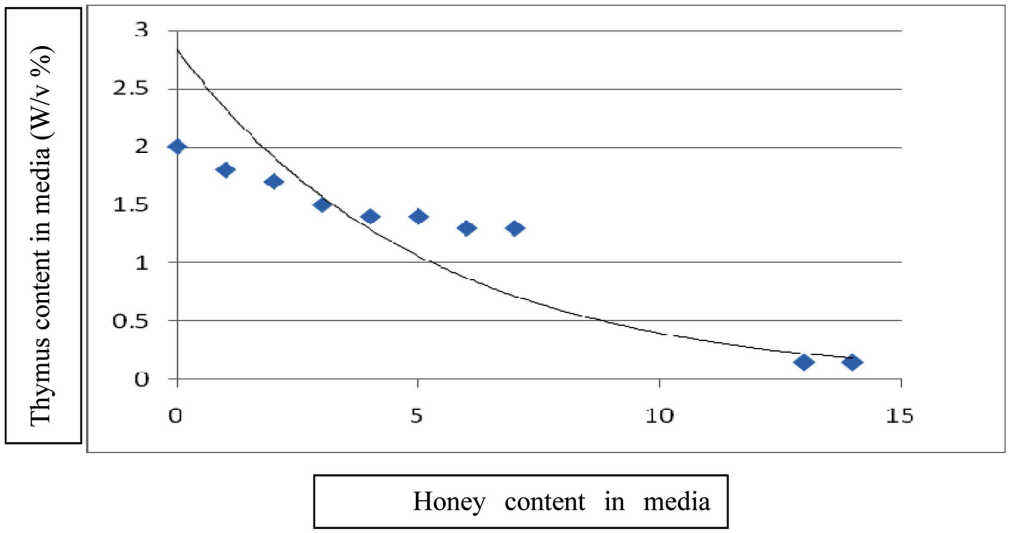

Fig. (3). Isobologram representing over additive effect of *Thymus* and honey against *Escherichia coli.*

in the specific media; the inoculums suspensions were obtained by taking five colonies from 24-hour cultures. The colonies were suspended in 5 ml of sterile saline (0.85% NaCl) and shaken for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to 1-5 x 10^6cfu/ml) using sterile saline.

MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC of honey and *Thymus* has to be determined by using an agar incorporation technique method. Honey was added in increasing quantities (Vol/Vol) into media for a final volume of 5 ml. *Thymus ciliatus* was incorporated into media by adding different weight of the powder. The mixture was shacked moderately with a vortex then poured into plates. The final volume was 5 ml. Majiscule plates were inoculated then incubated at 37°c for 24hours. The MIC was determined based on the lowest concentration of honey and *Thymus* that inhibited the growth of tested organism.

MINIMUM SYNERGISTIC INHIBITORY CONCENTRATION (MSIC)

In aseptic condition volumes of honey were mixed with weights of the powder of *Thymus ciliatus* lower than the MIC determined in the first step and then incorporated with

Mueller-Hinton media.The mixture was shacked moderately and poured into plates, then standard inoculums of 0.5 McFarland of bacterial strain were inoculated and the plates were incubated at 37°c for 24 hours.

RESULTS

Table **1** shows the MIC of honey and powder of *Thymus ciliatus* against the tested strains when used separately. Tables **2**, **3** and **4** indicate the synergistic effect of honey and thyme powder when used jointly. Fig. (**1**) and (**3**) shows an over additive action where as Fig. (**2**) indicates a simple additivity.

DISCUSSION

It has been demonstrated in many studies that honey has antibacterial effect, attributed to its high osmolarity, low pH, hydrogen peroxide and presence of other uncharacterized compounds [21]. The major antimicrobial properties are correlated to the hydrogen peroxide level which is determined by relative levels of glucose oxidase and catalase [22] whereas the non-peroxide factors that contribute to honey antibacterial and antioxidant activity are lysozyme, phenolic acids and flavonoids [23]. The powder of *Thymus ciliatus* has shown a strong antibacterial effect; this activity couldbe due to that the total powder contains all active components which may act synergistically against bacteria. It has been reported that carvacol and thymol which are the main compounds of *Thymus* have antibacterial properties [24, 25]. In a recent study carried out in Morocco, Amarti *et al*. [26] found that the yield of essential oils in *Thymus ciliatus* was 1.2%, and thymol represents 44.2% of this oil. Dorman *et al*. [27] demonstrated that thymol have a wide array of antibacterial activity against 25 tested bacterial strains. Another study realized by the World Health Organization [28] showed that this compound (thymol) has an important antifungal and antibacterial activity against many microbial strains such as *Aspergillus* sp., *S. aureus* and *E. coli*. Lambert *et al.* [29] and Juven *et al.* [30] explained this phenomena by the fact that thymol binds to the membrane protein and increases the permeability of bacterial cell membrane. Helander *et al.* [31] attributed the thymol antimicrobial action to its phenolic character, which can cause membrane-disturbing activities. Other studies suggested that this volatile compound was responsible for the inactivation of an enzyme implicated in syntheses of structural constituents [32]. Our results suggest that the use of thyme and honey together acted synergistically against the tested bacterial strains .The synergistic effect of honey and other compounds have been reported by many studies done by Boukraâ *et al*. [33-35].

CONCLUSION

The extensive use of antibacterial chemicals in medical area has led to the selection of resistant bacterial strains. So, to overcome this problem, it is necessary to find out alternative medicines that could be efficient and safe for use. *Thymus ciliatus* and honey are natural products which may be used jointly to treat superficial infections.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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None declared

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