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CHEMICAL COMPOUNDS FROM THYMUS VULGARIS AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT: This paper aims at highlighting the antibacterial activity of thyme (Thymus vulgaris) essential oils against the Staphylococcus aureus strain isolated from the nasal secretion of an anonymous patient, by employing the Kirby-Bauer method, and at establishing their chemical composition through GC MS methods. The oils were made from plants industrially grown in several geographic areas.

The measurements taken showed that thyme oils contain thymol (between 32% and 41.5%), and that the antibacterial action of thyme depends both on the provenance of the culture from which the essential oil was extracted and on the concentration of thymol in the oil, as well as on the sensitivity of the isolated strain.

KEY WORDS: Thymus vulgaris, oil extracts, antibacterial effect, Staphylococcus aureus

1. INTRODUCTION

Both modern microbiology and the pharmaceutical industry must face a real problem: the increasing resistance of microorganisms to the therapeutic agents available. Microorganisms have a high capacity to adapt to environment conditions, including antibiotics. As their resistance becomes more and more visible, efforts are made to synthetize new classes of antimicrobial agents. Resistance to antibiotics has become evident shortly after penicillin was widely introduced in therapeutics. Basically, this is the "price" that humans must pay in their continuous fight against microorganisms. Nowadays, general infections with S. aureus are treated with antibiotics. The global problem that medicine faces regarding microorganisms' resistance to antibiotics is well known and is a result of the fact that these are taken in excess, in a wrong or improper way, allowing bacteria strains to become resistant to antibiotics more often. In this sense, in the case of S. aureus, some strains are Methicillin-resistant; these strains are resistant to common antibiotics, such as penicillin, oxacillin, amoxicillin and methicillin. To avoid this problem, as well as the related or secondary issues that may arise after administering antibiotics to patients and in the ecosystem, through this study we propose increased attention to plant-based essential oils which have strong antibacterial effects, i.e. T. vulgaris (thyme) essential oils against S. aureus (Staphylococcus aureus); nevertheless, we recommend that these are used in accordance with the results of this study.

Several studies conducted in the field established the adjuvant qualities of essential oils in basic treatments of infections caused by Staphylococcus aureus, all the while leading to the reconsidering of global problems caused by antibiotic consumption. On the other hand, the increased frequency of Staphylococcus aureus was also taken into account, especially in the samples collected from nasal secretions (Fadila et al.,2016, Ghaleb et al., 2008).

In this context, we mention that staphylococci are among the most important pathogens found in nosocomial infections. Moreover, they are often identified in nasal secretion samples. According to the medical laboratory procedure, when S. aureus is found in such samples, an antibiogram is done to determine the antibiotics to which it is resistant and sensitive respectively, in order to clearly identify the antibiotic to be administered to the patient for an efficient treatment, but alternative solutions can also be offered, such as, in this case, essential oils having strong antibacterial effects.

S. aureus develops on the mucous membrane of nasal vestibules, as well as on the epidermis, in hair follicles. Besides, they colonise the mucosa of cavity organs communicating with the exterior, such as the colon and the vagina. It can be transmitted by direct contact, as it happens in most cases, or in other, much rarer ways in the case of certain infections, but this paper shall not touch upon these. However, should the bacteria reach the bloodstream or the surface of internal tissues, it may cause infections with extremely serious consequences (Tracey et al. 2017,)

Among others, the microbiological analysis of the samples taken from nasal swabs is recommended in order to detect whether the patient carries S. aureus, as advised by the epidemiologist. The carriage rate of this microorganism is 30% among healthy individuals and can reach as high as 70% in the case of laboratory and hospital staff (Buiuc 2008,)

Thymus vulgaris The medicinal properties of thyme mainly stem from essential oils extracted through steam distillation of fresh flowers and leaves. The main constituents of thyme essential oils are alpha-thujone, alpha-pinene, camphene, beta-pinene, para-cymene, alpha-terpinen, linalool, borneol, beta-caryophyllene, thymol and carvacrol. The chemical composition of thyme oil varies a lot (Rota et al., 2008, Romeo et al., 2008). From an antibacterial perspective, the chemotype "thymol" (2-isopropyl-5-methylphenol) is the most important, as it is the main active compound. Its proportion in this oil is about 40-50% and it is the main compound responsible for the antimicrobial action of this essential oil (Mancini et al., 2015, MardafKan et al., 2015, Hashmi et al., 2013, Khadir et al., 2013). Thymol is a monoterpene phenolic compound, an isomer of carvacrol (Prasanth 2014, Maher et al. 2011, Al Asmari et al., 2017, Maher et al., 2011, Mancini et al., 2011).

Thyme essential oil has a lot of beneficial features and people use it to improve peripheral circulation, as it stimulates blood stream to extremities and strengthens the immune system. This protects the heart and reduces the risk of blood clots. Some volatile compounds in thyme oil, such as camphene and alpha-pinene, can strengthen the immune system, thanks to their antibacterial and antifungal properties (Al Asmari et al., 2011, Firas et al., 2008)

Caryophyllenes and camphenes, together with some other components in thyme essential oils, inhibit bacteria growth inside and outside the body.

This is especially beneficial in treating bacterial infections, such as colitis, renal colic, bacterial infections of the reproductive organs and the urethra, of the intestines and the respiratory system, as well the outer exposure of wounds (Masoum et al., 2015).

2. MATERIALS AND METHODS

5 samples of thyme essential oils from: Greece, Italy, Hungary, China, India, noted: UTV1, UTV2, UTV3, UTV4, UTV5. To extract the oil, we used the aerial parts of the plant, which is harvested during blossoming. These undergo an 8-hour steam distillation, from which 0.3-0.7% volatile oil is obtained.

Identification and quantification the antimicrobial chemical compounds

The concentrations of thymol, α-terpineol and y-terpinen were determined through the GS-MS method (Miladi et al., 2013) optimised by Al-Asmari et al., 2017, which uses an injector temperature of 250oC, and a column temperature of a 60oC for 2 minutes, followed by a progressive increase by 2oC/minute until reaching 250oC. The oils were previously diluted with hexane, injected in the capillary column, and the mass spectra obtained were compared with the corresponding standards.

Antimicrobial activity

Essential oil was dissolved in DMSO (dimethyl sulfoxide) and sterilised by filtration with 0.22 µm Millipore filters. We then conducted antimicrobial tests, using 100 µl of suspension containing 1x 106 colony-forming units (CFU)/ml of bacteria determined through the analysis of their spread number in an environment of nutrient blood agar (NA). We placed (6 mm) disks in the inoculated agar, then using a pipette, we added 100 µl essential oil. The diameter of the inhibition zone (DIZ) was measured 24 hours after incubation and the DMSO was used as negative control.

The Kirby-Bauer Method (Bauer et al., 1966)

The method is based on the diffusion of the bactericide substance in the growth medium, which was inoculated with the strain under analysis, in a progressively decreasing concentration. Thus, a minimum inhibitory concentration is reached, which is to be semi-quantitatively determined by measuring the diameter of the inhibition zone of the microorganisms' growth around the disk impregnated with the bactericide substance.

Subsequently, the disks to be tested are placed at 15 mm distance from the edge of the plate leaving 30 mm between them, so as to avoid their interfering and to be able to correctly ascertain the diameter of the inhibition zone. The disks can be placed manually, using sterile tweezers, or via automated dispensers, which allow for faster work and stricter sterility control. The thus prepared plates are incubated in a thermostat at 370C for 24 hours. After incubation, using a millimetre ruler, we measure the diameter of the diameter of the inhibition zone of the microbial growth; this inhibition is directly proportional to that microorganism's in vitro sensitivity/resistance to the tested antibiotics - the minimum inhibitory concentration. We must mention that in some clinical situations, a certain microorganism's in vitro sensitivity can differ from its in vivo sensitivity, which leads to serious difficulties in the drawing up of the therapeutic plan (Bonev et al., 2008, Sokovic et al., 2010)

3. RESULTS AND DISCUSSIONS

Nowadays, due to their accuracy and sensitivity, GC-MS methods are the best ways to identify and quantify antibacterial volatile compounds.

Following the results obtained, we identified three compounds having antibacterial effect, i.e. thymol, α-terpineol and y-terpinen. As shown in Figure 1, thymol reached values between 32% and 41.52%. Higher values were determined in the case of oils UTV4 and UTV5, from China and India. The lowest amounts of thymol were found in oils extracted from plants cultivated in Greece and Italy (UTV1 and UTV2, reaching 34.21% and 31.11%).

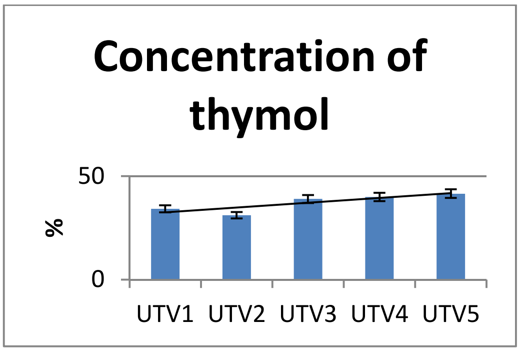

Figure 1. Thymol concentration in the 5 samples of essential oils from Thymus vulgaris from Greece, Italy, Hungary, China, India.

Figure 2 shows that the values of α-terpineol oscillate between 0.19%-0.21% and 0.31%. Maximum values were determined in sample UTV4 - 0.31%, while the lowest 0.19% and 0.21% were found in oils UTV2, UTV1. In the case of oils UTV3 and UTV5, α-terpineol reached 0.27% and 0.29%.

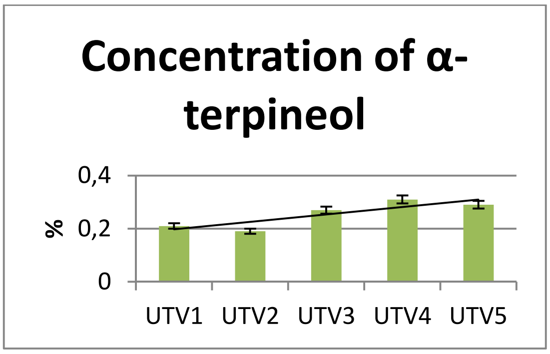

Figure 2. α-terpineol concentration in the 5 samples of essential oils from Thymus vulgaris from Greece, Italy, Hungary, China, India

Another valuable component of the antibacterial system is γ-terpinen. This compound reached around 1%, oscillating very little between samples. Thus, we found a minimum value of 0.92% y terpinen in sample UTV1 and a maximum of 1.11% in sample UTV3. Similar values were recorded in samples UTV4 and UTVS: 1.09%, respectively 1.05%.

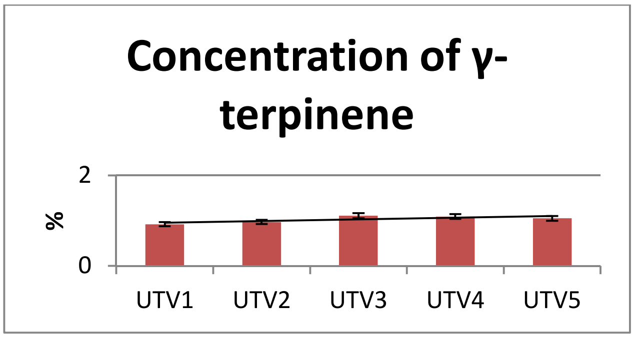

Figure 3. γ-terpinene concentration in the 5 samples of essential oils from Thymus vulgaris from Greece, Italy, Hungary, China, India

To establish the antibacterial activity of essential oils made from Thymus vulgaris against the Staphylococcus aureus strain, we used the Kirby-Bauer method; results can be consulted in Figure 4.

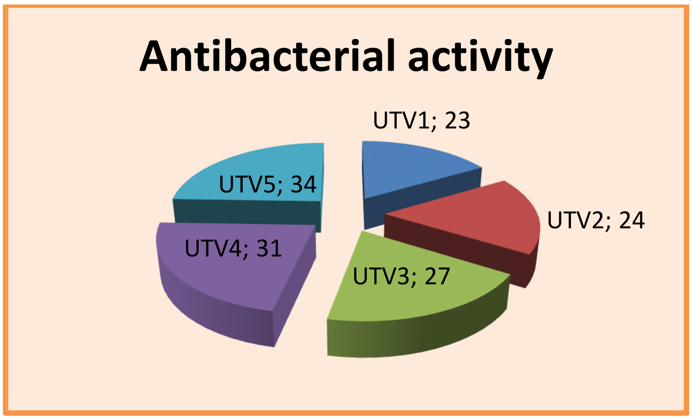

Figure 4. Antibacterial activity (mm) of essential oils Thymus vulgaris from Greece, Italy, Hungary, China, India against the Staphylococcus aureus strain

According to our measurements, the essential oils from India and China (UTV4, UTV5) have inhibition values of 31-34 mm, followed by the oils from Europe (UTV1, UTV2, UTV3) which recorded values between 23, 24 and 27 mm. The different sizes of the inhibition zones can be explained by the origin of the plant from which the oil was extracted, even if the same extraction scheme was followed. Pedoclimatic conditions can affect the accumulation of volatile compounds, which means that they will always vary. The Staphylococcus aureus strain reacted to the antibacterial action of the essential oils in all cases, showing sensitivity.

4. CONCLUSIONS

High amounts of the volatile compounds thymol, α-terpineol and y-terpinen were found and by identifying them we obtained visible antibacterial effects against the Staphylococcus aureus strain. The values determined proved that these compounds within Thymus vulgaris essential oils can vary depending on the origin and on the environmental factors of the place where the plants grew. The antibacterial action of essential oils on the Staphylococcus aureus strain leads us to the conclusion that these can be used as a cure, especially in cases in which, because of various medical reasons, antibiotics cannot be administered.

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