SUMMARY

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In the recent years a lot of species of lavender has appeared on the national market. These varieties can differ in chemical composition, and therefore in pharmacological properties. Despite the use of this raw material (flowers) in medicine and cosmetic as well as in food industries, chemical composition of various varieties and morphological parts of the plant is poorly understood. There is also not enough researches regarding their biological activity. In addition, it should be emphasized that the knowledge of phytochemistry and usage of leafy stalks is very limited due to the fact that the described pharmacopoeial material are only lavender flowers (*Lavandula Flos*), that are highly recommended for the isolation of essential oils.

The dissertation presents the results of phytochemical studies on biologically active substances in lavender (*Lavandula angustifolia*). Two varieties, the Blue River (BR) and the Ellagance Purple (EP), both the flowers (F) and the leafy stalks (UL) were analyzed. The plants were derived from experimental cultivation of the Department of Horticulture, West Pomeranian University of Technology in Szczecin. The varieties were compared with lavender flower from Herbal Plant 'Kawon'(KAW), available on the market in unknown variety.

One of the aims of the study was to determine the diversity of phytochemical composition in the following groups of active substances: essential oils (EO), phenolic acids, flavonoids, tannins, sesquiterpenic acids and microelements. All analyses were performed using both classical and modern spectroscopic and chromatographic methods: atomic absorption spectroscopy (AAS), flame photometry (FP), UV/VIS, high performance liquid chromatography (HPLC), ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC-ESI /MS/MS) and gas chromatography coupled with mass spectrometry (GC/MS).

It was found that the chemical composition of *L. angustifolia* is characterized by variability depending on the variety and anatomical parts of the plant. The results of analysis confirmed significant differences in the percentage of essential oils between the varieties as well as between flowers and leafy stalks. Moreover, in the case of essential oils from flowers significant

differences occurred in their percentage in the studied varieties of lavender; smaller differences were between year 2013 and 2014; in all studied cases with the use of the Deryng apparatus a higher content of essential oils was obtained than with the use of the Clevenger apparatus.

It was shown that the average content of essentials oils was the highest in flowers of Ellagance Purple (1.86 ± 0.06%) and the lowest in leafy stalks of Blue River (0.45 ± 0.14%). The results of the statistical analysis confirmed the statistically significant differences in the percentage of essential oils: between flower and leafy stalk, in particular years of experiment, with the use of Deryng or Clevenger apparatus. Nevertheless the type of distillation apparatus had a minimal impact on the percentage of essential oils in comparison to the other tested factors. In addition, it was proved that the type of the apparatus did not also have a significant impact on the qualitative composition of essential oil.

The results indicate that the essential oils obtained from all tested varieties of *L. angustifolia* contain similar main components (linalool, linalyl acetate, lavandulol acetate, borneol, eucalyptol, α-terpineol, geraniol acetate), however, there are differences in the qualitative composition of the compounds occurring in low concentrations, which may have a significant impact on the biological properties of essential oils. Chromatographic analysis (GC/MS) showed that the isolated essential oils are characterized by different percentages of the compounds and sometimes by different qualitative chemical composition, depending on the variety, morphological part of the plant and the year of harvest. Most of the identified compounds in all isolated essential oils can be classified as monoterpenes (oxygenated monoterpenes). By analyzing the experimental data concerning oxygenated monoterpenes it was found that there are significant differences between varieties of lavender from year 2012 and 2013. However, there were no significant differences in content of oxygenated monoterpenes between year 2012 and 2014 and with the use of the two types of apparatus.

Chromatographic analysis (HPLC) indicated that the investigated plant materials differ with the presence of phenolic acids. Three phenolic acids: rosmarinic, ferulic and caffeic were found and their content was determined. Rosmarinic acid was present in the highest content, ranged from 1.5 ± 0.3 mg /g d.m. in the Ellagance Purple leafy stalks to 9.7 ± 0.5 mg /g d.m. in the commercial lavender flowers ‘Kawon’.

The tested herbs also differ in content of flavonoids. Spectrophotometric analysis (UV-VIS) showed that the total flavonoid content ranges from 86.0 ± 2.0 mg /100 g in the Ellagance Purple flowers to 108.0 ± 20.0 mg /100g in the leafy stalks in the commercial lavender. The content of

flavonoids in leafy stalks ranges from 341.0 ± 27.0 mg /100 g in the Ellagance Purple to 352.0 ± 31.0 mg /100 g in the Blue River. It should be noted that leafy stalks contained three times more flavonoids than flowers. UPLC quantification, based on external calibration method, showed the presence of following flavonoids in the examined plant material: apigenin, apigenin glucoside, apigenin glucurono-glucoside, luteolin glucuronide, luteolin diglucuronide, luteolin p-kumaroil-glucurono-diglucoside and quercetin p-kumaroil-glucurono-diglucoside. Quantitative studies confirmed that the lavender leafy stalks had higher accumulation of flavonoids.

In case of the analysis of tannins it was found that there is no clear dependency on the particular part of the plant. On the basis of spectrophotometric analysis (UV-VIS) it was found that the highest content of tannins was present in the Ellagance Purple stalks (0.48 ± 0.002%), whereas the lowest content was observed in commercial lavender flowers (0.15 ± 0.002%).

Chromatographic analysis (HPLC) of sesquiterpenic acids confirmed that the tested herbal materials vary in terms of their content. Generally, higher concentrations of these acids were observed for samples of flowers. The highest content of valerenic acid was found in the Ellagance Purple (1.5 ± 0.06 mg /100g), and the lowest in the Blue River (0.75 ± 0.02 mg /100 g). On the contrary, the Blue River flowers had a ten times higher content of acetoxy valerenic acid (65.8 ± 1.51 mg /100 g) in comparison to the Ellagance Purple flowers (6.5 ± 0.18 mg /100 g), which was also observed for leafy stalks.

With the use of atomic absorption spectrometry (AAS) and flame photometry (FP) it was determined that the plant material contains 8 elements, in concentration depending on the plant variety and morphological part.

The efficiency of various extraction techniques of lavender was investigated. It was found that the yields rise consecutively for the following methods: maceration < sonification < extraction in a Soxhlet apparatus. Duration of the extraction had an impact on the efficiency. The lowest efficiency level of 2-3% was achieved as a result of two hours maceration and shaking maceration, however it was not observed that shaking had significant impact on the efficiency of the process. While all attemps to ten-hours extraction in Soxhlet apparatus gave the highest yields (approx. 16-20%). There was no direct correlation between the efficiency of the process and the type of raw material which was extracted.

The chromatographic analysis (GC/MS) of obtained extracts allowed to identify three main chemical components of the extracts. In most cases, these were coumarin and herniarin.

However, the flower extracts obtained by two-hour maceration contained linalool acetate. There was no direct correlation between the efficiency of the extraction process and relative percentages of compounds and variety or morphological part of extracted material.

The antioxidant activity was evaluated in relation to the lavender flowers essential oils of the Blue River, the Ellagance Purple and the commercial lavender. The activity of decoctions and infusions of flowers and leafy stalks was also determined. The assessment was performed with the use of spectrophotometric method with a stable radical DPPH. As a control was used a synthetic antioxidant – BHA, which deactivates the radical DPPH at level of 99.0%. The essential oils, infusions and decoctions showed antioxidant properties, however this activity was diverse. Essential oils and decoctions showed stronger antioxidant properties than infusions. Among analyzed flower extracts the highest ability to deactivate radical DPPH was determined for essential oil (76.6 ± 0.021%) and decoction (72.5 ± 0.001%) obtained from commercial lavender flowers, which contained 4.5 times more phenolic acids than the material with one of the lowest capacity to inactivate the radical and also the lowest content of these acids (the Blue River). In the lavender flowers of commercial origin the highest concentration of rosmarinic acid was found, which might affect the antioxidant activity. The highest antioxidant activity of leafy stalks extracts was found for the Ellagance Purple (decoction 70.5 ± 0.005%, and infusion 61.0 ± 0.004%), in which the highest accumulation of polyphenolic bioactive substances, such as flavonoids and tannins, was determined.

In developing new therapeutic strategies, except from proposals regarding an independent use of natural compounds belonging to defense systems of plants and animals, antibiotics or antiseptics are considered as additional components to be used together with them. In the literature there are no reports concerning the interaction between lavender oils and synthetic antibiotics such as gentamicin or fluconazole. This was a reason for undertaking studies on the potential synergism of antimicrobial drugs and essential oils, and one of their main ingredients - linalool. Moreover, the impact of synthetic preservatives (parabens mixture) was also examined. The interaction of the isolated lavender oils with synthetic antibiotics, gentamicin and fluconazole, on selected strains of bacteria and fungi was confirmed in this research, which is a novelty. The antimicrobial activity studies included the evaluation of the impact of the essential oils from flowers of the Blue River, the Ellagance Purple and the commercial lavender as well as leafy stalks of the Blue River and the Ellagance Purple on cells of reference strains of bacteria: Gram-positive (*S. aureus*), Gram-negative (*P. aeruginosa*) and fungus cells (*C. albicans*). In addition, the activity of essential oils on clinical methicillin-resistant *S. aureus*

strain was evaluated. In the first stage of the study the lowest concentrations of essential oils, linalool and parabens inhibiting the growth of microorganisms (MIC) were determined using the dilution method. Next, their interaction with antibiotics was tested using a checkerboard method and the FIC factors (fractional inhibitory concentrations) were calculated.

Among the isolated essential oils the highest antimicrobial activity was observed for the essential oil of the Blue River flowers. This oil was the most active against strains of *S. aureus*, exhibiting drug resistance. The antimicrobial properties of this oil may be related to its qualitative composition, i.e. the content of α-santalene – a compound with antibacterial properties which was not present in other flower oils. This component also occurred in the composition of essential oils of leafy stalks, possessing low levels of linalool. It should be emphasized that linalool has been regarded so far as a component conditioning antimicrobial activity of lavender essential oils.

The studies confirmed the hypothesis regarding the synergism (ΣFIC 0,076–0,403) between isolated essential oils and gentamicin and fluconazole (against *S. aureus* and *C. albicans*), which is a valuable conclusion for further *in vivo* tests and justify the validity of using natural and synthetic substances together. It should be emphasized that in any combination of oils, linalool and parabens with antibiotics antagonism was not observed.

Moreover, attempts to apply the essential oils for preservation of emulsions and cosmetic ointments were done. It was found that the essential oil from the Blue River flowers, used at a concentration of 2% (v/v) for the preservation of cosmetic emulsions, as well as the essential oils from the Ellagance Purple flowers, at a concentration of 0.5% (v/v) for the preservation of ointments, may be an alternative to synthetic preservatives in order to maintain the microbial purity of recipes.

This work has an interdisciplinary character – above all it provides practical value regarding to the application studies and therefore it could be used to improve safety of cosmetics and medicines. The data can be also used to prepare a monograph. The obtained results of the research indicate new possible ways of using plant biologically active substances and represent a significant contribution to the development of technology of cosmetics and medicines.

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