SUMMARY

of the doctoral thesis entitled:

EXPLORATION OF THE VALUATION POTENTIAL OF A NEW VARIETY OF LAVENDER

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The *Lavandula* genus, known for its synthesis of bioactive compounds, has witnessed a notable surge in production over the past 15 years. This escalation underscores the industrial relevance of *Lavandula* in pharmaceutical, perfumery, and food sectors. *Lavandula*'s bioactive constituents find applications in medicinal formulations, perfumery products, and culinary creations, affirming its significance as a versatile botanical resource with broad scientific and commercial implications.

Romania boasts an endemic lavender species, named *George 90*, indexed in April 2017 in the Official Catalog of Varieties. Analyzed by the State Institute for Testing and Registration of Varieties, *George 90* exhibits distinct morphostructural properties setting it apart from other *Lavandula* species. Characterized by sprawling growth, large size, and unique features, this lavender variety has green foliage with subtle gray tones and lacks leaf edge incisions. The flowering stem is long and flexible, non-pubescent, with numerous side branches. The ear is wide and lengthy, featuring a cylindrical shape with spaced vertices and numerous flowers, each adorned with a greenish, pubescent calyx and a purple corolla. *George 90*'s recognition in the Official Catalog of Varieties highlights its uniqueness within the *Lavandula* genus.

The doctoral thesis is organized into nine chapters. The initial **chapters, I and II**, offer an in-depth exploration of lavender species cultivation techniques, concurrently delving into the current state of knowledge. This includes information extracted from literature on the chemical composition, uses, and bioactive properties of well-known lavender species. Subsequent **chapters, III to IX**, contribute original insights by meticulously detailing the chemical composition of *Lavandula angustifolia, Lavandula latifolia*, and the Romanian species *George 90*. Moreover, these chapters unveil, for the first time, pertinent information regarding the biological properties of the identified compounds in the study.

The **aim of the doctoral thesis** was to analyze *the potential for valorisation of a new lavender variety - George 90*, compared to the known species - *L. angustifolia* and *L. latifolia*. To achieve the proposed goal, comparative cultures were established and extractions of the active principles from the lavender species were carried out. Both the extracts from the biological material and the obtained oils were analyzed and the biological properties of the biologically active compounds identified in the studied lavender species were highlighted. Tests have also been conducted to evaluate the antitumor potential of lavender's active compounds.

The outlined ***specific objectives*** were as follows:

- Establishment of comparative cultures, phenological evaluation of studied species and demonstration of genetic diversity of lavender cultivar: *L. angustifolia*, *L. latifolia* and *George 90*.
- Extraction of essential oils from lavender species: *L. angustifolia*, *L. latifolia* and *George 90*, by using the microwave-assisted extraction method;
- Performing the extraction of polyphenolic compounds from the lavender species *L. angustifolia*, *L. latifolia* and *George 90*, by using the microwave-assisted extraction method;

- Separation and identification of bioactive compounds from lavender species *L. angustifolia*, *L. latifolia* and *George 90*, by using advanced analytical techniques;
- Evaluation of the antioxidant activity of the bioactive compounds from the lavender species *L. angustifolia*, *L. latifolia* and *George 90*, by different microspectrophotometric methods, such as the DPPH, $ABTS^{\bullet+}$ and FRAP methods;
- Evaluation of the antimicrobial activity of bioactive compounds from lavender species *L. angustifolia*, *L. latifolia* and *George 90*, using *in vitro* inhibition methods, including the diffusimetric method, determination of the minimum inhibitory concentration and determination of the minimum bactericidal/fungicidal concentration;
- Evaluation of the antitumor activity of the bioactive compounds from the lavender species *L. angustifolia*, *L. latifolia* and *George 90*, using *in vitro* methods for evaluating cell viability, such as the MTT test.

The originality of the doctoral thesis is emphasized through the exploration of the Romanian lavender species *George 90* and the exhaustive analysis conducted on the remaining two lavender species, *Lavandula angustifolia and Lavandula latifolia*. This uniqueness is reinforced by the comprehensive examination of the chemical composition of these three lavender species, coupled with an analysis of the biological potential inherent in the identified compounds within the study.

Chapter III, titled "***Lavendula sp. comparative culture establishment, phenological analysis and genetic diversity***", details the initiation, progression, and manifestation of genetic diversity within the lavender culture. In terms of plant height, a five-year observation indicates that the Romanian George 90 species reached 129 cm, insignificantly lower than the *Lavandula latifolia* species (131 cm) but markedly distinct from the *Lavandula angustifolia* species (75 cm). Compared to the mean value, the *Lavandula angustifolia* species exhibits a 37 cm shorter stature. The length of inflorescences in the initial year of cultivation (2017) ranges from 10 cm (*Lavandula angustifolia*) to 13 cm (*Lavandula latifolia*). Subsequent years show negligible differences, with the Romanian species *George 90* displaying an average inflorescence length of 12 cm.

The species *George 90* has an average length of inflorescences of 12 cm. The average length of the floral stem was 70.6 cm in the Romanian species George 90, 70 cm in *Lavandula latifolia*, and 43 cm in *Lavandula angustifolia*. The number of whorls on the flower spike exhibited annual and varietal variations. For *George 90*, it ranged from 86 (2020) to 92 (2018). In *Lavandula angustifolia*, the range extended from 70 (2018, 2020, 2021) to 72 (2017 and 2019), while in *Lavandula latifolia*, it spanned from 78 (2017) to 84 (2019 and 2021). *George 90* demonstrated a superior average vertex count per ear, surpassing other varieties with 4.4 compared to 2.4 in *Lavandula latifolia* and 12.4 in *Lavandula angustifolia*.

Inflorescence yield displays variation influenced by both the year of observation and the specific lavender variety. The lowest recorded yields occurred in the initial year (2017), ranging from 1716 kg/ha (*Lavandula angustifolia*) to 3218 kg/ha (*George 90*). Conversely, the highest yields were observed in the fifth year (2021), maintaining the same variety order (6435 kg/ha – *Lavandula angustifolia* and 18590 kg/ha - *George 90*). Average yields rank the experimental variants as follows: *George 90* – 11283 kg/ha, *Lavandula latifolia* – 7004 kg/ha, and Lavandula angustifolia - 4403 kg/ha. Deviations from the variant mean (13583 kg/ha) are notably positive for *George 90* and *Lavandula latifolia*, while significantly negative for *Lavandula angustifolia* (DL5% = 465.14 kg/ha; DL 1% = 618.68 kg /ha, LD 0.01% = 801.71 kg/ha). This trend is consistent when evaluating the amount of essential oil extracted per unit area.

The dendrogram analysis underscores the segregation of samples into two discernible subgroups: one comprising *Lavandula latifolia*, *George 90*, and lavender from the market, and the other encompassing *Lavandula angustifolia* and *Lavandula angustifolia* Blue scent LV-BS. The utilization of RAPD markers facilitated the elucidation of genetic relationships among the tested samples, demonstrating utility in identifying specific markers for lavender species. The outcomes revealed a 86.75% polymorphism across the five tested samples, signifying a notable degree of variability, attributed in part to the predominantly outdoor cultivation practices of lavender species.

Chapter IV, titled "***Extraction of bioactive compounds from investigated lavender species***," entails the planned sampling and preparation of plant material, as well as the extraction of essential oil and polyphenolic compounds. The lavender plant material came from Buftea, respectively from Suceava. Sampling, a pivotal phase for achieving the stipulated objectives, took place in June 2022, coinciding with the maturity of lavender species and the peak concentration of bioactive compounds. Subsequently, the sampling stage was succeeded by the preparation of plant material and its subsequent analysis.

To procure lavender essential oil enriched with bioactive compounds, the advanced Microwave-Assisted Extraction (MAE) method was employed, with a conventional Hydrodistillation (HD) extraction serving as a comparative technique. Significantly higher yields of essential oil were observed using the MAE extraction method compared to the HD extraction method. Lavender species *George 90* exhibited the highest extraction yield for essential oil, regardless of the extraction method employed, showcasing its potential in essential oil production.

To optimize the extraction yield, the Microwave-Assisted Extraction (MAE) method was employed for obtaining polyphenolic-rich extracts from the aerial components of lavender species, namely *Lavandula angustifolia*, *Lavandula latifolia*, and *George 90*. The extraction utilized polar solvents, such as ethanol (EtOH) and methanol (MeOH). The extraction process was conducted at a predetermined temperature of 70 °C, chosen based on the boiling points of the solvents (ethanol and methanol). In this extraction procedure, solvents including EtOH 50%, EtOH 70%, and MeOH 50% were employed to enhance the heating efficiency within the vessel, thereby facilitating the extraction process. The presence of water, along with EtOH or MeOH solvent, contributed to the overall effectiveness of the extraction.

To assess the impact of the plant material/solvent ratio on polyphenolic compound extraction, two ratios, 1:20 (m/v) and 1:40 (m/v), were established. Two distinct plant material sizes, labeled as coarse (G1) and very fine (G2), were employed in the investigation. Through Microwave-Assisted Extraction (MAE), a total of 12 extracts were obtained for each lavender species (*Lavandula angustifolia*, *Lavandula latifolia*, and *George 90*). Notably, 50% MeOH exhibited a significantly superior extraction of polyphenolic compounds compared to 50% EtOH. In alignment with both MAE and Hydrodistillation (HD) methods for essential oil extraction, *George 90* lavender species consistently yielded the highest quantity of polyphenolic

In ***Chapter V***, titled "***Analysis of essential oils extracted from the inflorescences of investigated lavender species***," the GC-MS/MS analysis is detailed. This analysis pertains to lavender essential oils obtained from *Lavandula angustifolia*, *Lavandula latifolia*, and *George 90* species, employing two distinct extraction methods: Microwave-Assisted Extraction (MAE) and Hydrodistillation (HD).

A total of 41 bioactive compounds were identified, all falling within the class of hydrocarbons and hydrocarbon derivatives. The efficacy of the Microwave-Assisted Extraction (MAE) method was reiterated, surpassing the Hydrodistillation (HD) method, as demonstrated by the richer concentration of bioactive compounds in the essential oils of *Lavandula angustifolia*, *Lavandula latifolia*, and *George 90* obtained through MAE extraction. In George 90 essential oils, both from MAE and HD extraction methods, the presence of camphor, a terpenoid known for its anti-inflammatory properties, was identified, akin to *Lavandula angustifolia* essential oils.

Chapter VI, titled "***Analysis of polyphenolic compounds present in the studied lavender species***," systematically explores the separation, identification, and quantification of polyphenolic compounds in the hydroalcoholic extracts of *Lavandula angustifolia*, *Lavandula latifolia*, and *George 90*. The hydroalcoholic extracts of the studied lavender species (*Lavandula angustifolia*, *Lavandula latifolia*, and *George 90*) were subjected to a thorough analysis, achieving an impressive 95% success rate in the separation, identification, and quantification of reference compounds. The extraction of bioactive compounds from the polyphenol class was carried out using Microwave-Assisted Extraction (MAE), employing the most effective parameters, namely EtOH 70%, plant material particle size G2, and a solvent material ratio of 1:40.

The hydroalcoholic extracts of *Lavandula latifolia* also demonstrated elevated levels of flavonoids, namely naringin, naringenin, and ellagic acid. Conversely, the hydroalcoholic extracts of *Lavandula angustifolia* showcased increased concentrations of various flavonoids.

Chapter VII, titled "***The antioxidant potential of bioactive compounds in investigated lavender species***," systematically assesses the antioxidant potential of the bioactive compounds inherent in the lavender species *Lavandula angustifolia*, *Lavandula latifolia*, and *George 90*.

The antioxidant activity of lavender extracts from *Lavandula angustifolia*, *Lavandula latifolia*, and *George 90* correlates with the presence of key polyphenolic compounds. In the assessment of antioxidant potential using the DPPH, ABTS●+, and FRAP methods, hydroalcoholic extracts of lavender obtained through extraction with EtOH 70% at a ratio of 1:40 (m/v) exhibited superior antioxidant potentiaLavandula Subsequently, lavender hydroalcoholic extracts extracted with MeOH 50% at a ratio of 1:40 (m/v) and those extracted with EtOH 50% at a ratio of 1:40 (m/v) demonstrated lower but appreciable antioxidant potential in sequential order.

The assessment of the antioxidant potential of lavender samples (essential oils and hydroalcoholic extracts) from *Lavandula angustifolia*, *Lavandula latifolia*, and *George 90*, conducted using the DPPH, ABTS●+, and FRAP methods, yielded results comparable to existing literature. However, their antioxidant potential is relatively weaker when compared to reference standards like trolox and ascorbic acid. Notably, across all methods employed for evaluating antioxidant potential (DPPH, ABTS●+, and FRAP) for both essential oils and hydroalcoholic extracts, the Romanian species *George 90* consistently exhibited the highest antioxidant activity, followed by *Lavandula angustifolia* and *Lavandula latifolia* lavender species, respectively.

Chapter VIII, entitled "***The antimicrobial potential of bioactive compounds present in the studied lavender species***," details the assessment of antibacterial and antifungal potential inherent in the bioactive compounds of *Lavandula angustifolia*, *Lavandula latifolia*, and *George 90* lavender species. The essential oils extracted from *Lavandula angustifolia*, *Lavandula latifolia*, and *George 90*, utilizing both MAE and HD extraction methods.

Lavender essential oils, extracted through both MAE (microwave-assisted extraction) and HD (hydrodistillation) methods, from *Lavandula angustifolia* (LA), *Lavandula latifolia* (LL), and the Romanian species *George 90* (G90), exhibited substantial antimicrobial efficacy against Gram-positive bacterial strains, including *B. subtilis and B. cereus*. The G90-MAE and G90-HD essential oils demonstrated robust antimicrobial potentials against B. subtilis and moderate potentials against *B. cereus*. Notably, the LL-MAE essential oil displayed the most potent antibacterial activity against *B. subtilis*, followed closely by G90-MAE. Meanwhile, the LA-MAE essential oil exhibited the highest antibacterial potential against *B. cereus*, with LL-MAE essential oil ranking second. Overall, all essential oils derived from *Lavandula angustifolia, Lavandula latifolia, and George 90* showcased significant antibacterial activity against the tested *Gram-positive* bacterial strains.

Both MAE (microwave-assisted extraction) and HD (hydrodistillation) methods yielded lavender essential oils from *Lavandula angustifolia* (LA), *Lavandula latifolia* (LL), and the Romanian species *George 90* (G90), demonstrating notable antifungal potential. The most robust antifungal activity was observed against the *A. brasiliensis* strain, followed by moderate antifungal potential against the *F. oxysporum* and *P. expansum* strains, respectively. Utilizing a 20 µL quantity of lavender essential oil from *Lavandula angustifolia, Lavandula latifolia, and George 90*, strong inhibitory effects were evident against the *F. oxysporum* strain. Notably, essential oils obtained through MAE extraction exhibited greater antifungal activity compared to those obtained through HD extraction for all lavender species.

Lavender essential oils *L. angustifolia*, *L. latifolia* and *George 90*, obtained by both MAE and HD extraction, showed antifungal potential. The strongest antifungal potential was obtained on the *A. brasiliensis* strain, followed by moderate antifungal potential on the *F. oxysporum* and *P. expansum* strains, respectively. When the amount of 20 µL of lavender essential oil *L. angustifolia*, *L. latifolia* and *George 90* was used, a strong inhibitory activity against the *F. oxysporum* strain was observed, on the other hand lavender essential oils

obtained by MAE extraction show more antifungal activity strong compared to lavender essential oils obtained by HD extraction.

Essential oils of lavender *L. angustifolia* and *George 90* showed strong antifungal potential against the *A. brasiliensis* strain, with a degree of inhibition of 100±0.0% for all amounts of essential oil tested (20 - 45 μL). When the amounts used were 20 μL and 25 μL, the degree of inhibition varied between 76.7±12.0- 97.24±2.8%, while when using larger amounts, the degree of inhibition is 100±0.0%.

For the *P. expansum* strain, the observed inhibition degree remained consistently at 100±0.0% when testing 45 μL amounts of lavender essential oils from *Lavandula angustifolia* (LA), *Lavandula latifolia* (LL), and the Romanian species *George 90* (G90), obtained through both MAE and HD extraction methods. This uniform inhibition suggests a notable natural resistance of the *P. expansum* strain. In contrast, the essential oil G90-MAE exhibited a 100±0.0% inhibition degree even at lower amounts (25 - 40 μL). When testing amounts between 25 μL and 40 μL, lavender essential oils from *Lavandula angustifolia, Lavandula latifolia, and George 90* (G90-HD) showed comparable inhibition degrees, ranging from 76.5±0.0% to 97.7±2.4%.

Presented in ***Chapter IX***, titled "***Antitumor potential of bioactive compounds present in lavender species***," presents the antitumor potential of the essential oils *of L. angustifolia*, *L. latifolia* and *George 90*, evaluated on the HeLa tumor cell line, using the MTT assay. This study indicated strong antitumor potential at low concentrations (1%), which means that *L. angustifolia*, *L. latifolia* and *George 90*essential oils contain different biologically active compounds and each compound enhances or modifies the antitumor potential.

Based on the specialized literature and the results obtained in the study, we can deduce that the antitumor potential of *L. angustifolia*, *L. latifolia* and *George 90* essential oils is closely related to the metabolism of the biologically active compounds present and their synergistic effect. Lavender essential oils have been reported to have antitumor potential on several tumor cell lines (Calu-3, U-373, PC3, MCF-7).

The **novel elements** of the doctoral thesis were:

1. Comparative analysis of the lavender species *L. angustifolia*, *L. latifolia* and *George 90* from the point of view of biological productivity and demonstration of the genetic diversity of the species *George 90*;
2. Utilizing advanced analytical techniques the chemical composition of lavender species, including *Lavandula angustifolia, Lavandula latifolia, and George 90*, was characterized;
3. Conducting in vitro inhibition methods, the biological potentials of lavender species, namely *L. angustifolia*, *L. latifolia*, and *George 90*, were evaluated. This assessment encompassed various aspects such as antioxidant, antibacterial, antifungal, and antitumor potentials

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