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ISOLATION, CHEMICAL CHARACTERIZATION AND ASSESSMENT OF PHARMACOLOGICAL ACTIVITY OF BIOLOGICALLY ACTIVE COMPOUNDS OF LAVANDULA ANGUSTIFOLIA

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Abstract
The article analyzes methods for the extraction, chemical characterization and pharmacological evaluation of bioactive compounds from Lavandula angustifolia. An integrated use of chromatographic and biological assays is proposed for standardizing essential oils and phenolic extracts.

Keywords: Lavandula angustifolia, essential oil, linalool, linalyl acetate, phenolics, GC–MS, pharmacological activity.

LAVANDULA ANGUSTIFOLIA O‘SIMLIGINING BIOLOGIK FAOL BIRIKMALARINI AJRATIB OLISH, KIMYOVIY TAVSIFLASH VA FARMAKOLOGIK FAOLLIGINI BAHOLASH

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Annotatsiya
Maqolada Lavandula angustifolia o‘simligining biologik faol birikmalarini ajratib olish, kimyoviy tavsiflash va farmakologik faolligini baholash usullari ilmiy manbalar asosida tahlil qilindi. Efir moyi va fenolik ekstraktlarni standartlashtirish uchun xromatografik hamda biologik sinovlardan kompleks foydalanish zarurligi asoslandi.

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Kalit so‘zlar: Lavandula angustifolia, efir moyi, linalool, linalil asetat, fenolik birikmalar, gidrodistillatsiya, GC–MS, UPLC–MS/MS, antioksidant faollik, antimikrob faollik.

INTRODUCTION

Lavandula angustifolia Mill. (Lamiaceae) is a perennial medicinal and aromatic plant originating from the Mediterranean region, widely used in the pharmaceutical, perfumery, cosmetic and food industries. The main pharmacognosic raw materials are its flowers and essential oil. Correct identification of medicinal plant raw materials, collection at a certain phenophase, proper drying and storage are important conditions for preserving biologically active substances [1, 2]. However, the chemical composition of products under the name “lavender” is not uniform: genotype, geographical area, soil-climatic conditions, collection phenophase, drying method and extraction technology significantly change the ratio of linalool, linalyl acetate, camphor and phenolic substances [3, 7].

It is not enough to explain biological activity only by the name of the plant. It is necessary to correlate the pharmacological effect with the chemical profile of a particular extract, dose, solvent, test model and control drug. Therefore, the aim of the study is to systematize the optimal methods for the extraction of volatile and polar biologically active compounds from L. angustifolia raw materials, characterize them using modern chromatographic-spectrometric methods, and substantiate a consistent scientific approach to assessing pharmacological activity. For standardization of raw materials, flower collections collected during the flowering period should be certified by a botanist and stored in a voucher specimen herbarium. The moisture content, foreign impurities, and ash content are determined based on pharmacopoeial approaches. The sample is dried in the shade at a temperature not exceeding 25–35 °C, and after grinding, it is stored in a container protected from light and moisture. The use of at least three independent samples of the same batch allows for statistical comparison of the results.

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Hydrodistillation for 2–3 hours in a Clevenger-type apparatus is a practical and reproducible method for obtaining essential oil. The obtained oil is dried over anhydrous sodium sulfate and stored in a dark glass container at about 4 °C; the yield is expressed as a percentage of the dry mass of the raw material. Microwave-assisted hydrodistillation can reduce time and energy consumption. Kırkıncı et al. compared conventional and microwave hydrodistillation products by GC–MS and showed that the choice of method affects the oil profile and bioactive potential of the distillation residue [3]. Low-temperature supercritical CO_2 extraction is promising in preserving thermolabile components, but requires high-pressure equipment and expensive validation.

For phenolic and flavonoid fractions, a 50–70% ethanol-water mixture is optimal from the point of view of safety, polarity, and subsequent pharmaceutical application. The ground raw material is extracted in an ultrasonic bath at a solid-solvent ratio of 1:10 or 1:20 for 30–60 min. Then, it is filtered, the solvent is removed under vacuum at a temperature not exceeding 40 °C, and the dry extract yield is calculated. In the work of Özderin, ultrasound-assisted separation and UPLC–ESI–MS/MS analysis allowed the identification of nine phenolic compounds in the flowers and leaves of L. angustifolia [4]. If fractionation is necessary, the crude extract is sequentially partitioned between hexane, ethyl acetate, n-butanol, and water. Hexane enriches lipophilic terpenes, ethyl acetate enriches medium-polar phenols, and butanol enriches glycosidated flavonoids.

Gas chromatography–mass spectrometry (GC–MS) is the main tool for the qualitative and quantitative control of the volatile fraction. Components are identified by comparison with a mass spectral library and by Kovacs retention indices calculated for the n-alkane series. Quantitative content is determined using GC–FID peak areas or an internal standard. Typical markers of L. angustifolia essential oil are linalool and linalyl acetate, and terpinen-4-ol, lavandulol, lavandulyl acetate, β-caryophyllene, 1,8-cineole, and camphor are also present [3, 7]. High levels of camphor or 1,8-cineole may indicate admixture with other Lavandula species or chemotype differences; therefore, relying on a single marker alone is not sufficient.

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Initial screening of polar extracts begins with the estimation of total phenols as gallic acid equivalents by the Folin–Ciocalteu method and flavonoids as quercetin equivalents by the aluminum chloride method. As these are non-selective integral indicators, individual compounds should be confirmed by HPLC–DAD or LC–MS/MS. Rosmarinic, caffeic, ferulic, p-coumaric, protocatechuic and vanillic acids; luteolin, apigenin, rutin and quercetin derivatives have been reported in L. angustifolia extracts [2, 4, 5]. Dobros et al. have shown that the total phenol and antioxidant activity of different extracts vary with solvent and plant part [5]. For final confirmation of the structure, high-resolution MS and $^{1}H/^{13}C$ NMR are used on compounds isolated by preparative chromatography.

Antioxidant activity should be measured by at least two complementary methods. DPPH and ABTS radical scavenging assays indicate the free radical scavenging capacity of the extract, while FRAP indicates the ferric ion scavenging capacity. Results are reported as IC_{50} or Trolox equivalents, with Trolox/ascorbic acid as a positive control. A correlation between total phenolic content and antioxidant activity is considered, but the correlation cannot be interpreted as a causal mechanism. The possibility of phenolic compounds being retained in distillation effluent also suggests that the effluent may be considered a secondary source of bioactivity [3].

The antimicrobial activity can initially be assessed by agar diffusion, but due to the poor solubility of the essential oil in water, it is advisable to provide a final conclusion by broth microdilution with minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration. Standard ATCC strains such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans, antibiotic/antimycotic positive controls and solvent controls are used. The emulsifier content for the lipophilic oil is kept constant. Possible mechanisms include disruption of membrane permeability of lavender oil, and interaction of linalool and other monoterpenes with the cell membrane of microorganisms [6, 7].

Anti-inflammatory potential is assessed in vitro by protein denaturation, lipoxygenase/cyclooxygenase inhibition, or NO, TNF-α, and IL-6 production in LPS-stimulated macrophages. Cytotoxicity is not assessed in parallel with the

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MTT or resazurin assay, as the reduction of inflammatory mediators may be confused with cell death. There is evidence that essential oil components have anxiolytic and sedative effects on GABAergic and glutamatergic transmission, the serotonin system, and voltage-gated calcium channels [7]. Animal experiments should only be conducted with ethical approval, randomization, blinding, and a predetermined dose. Evidence for clinical use cannot be extrapolated from in vitro results.

The analysis showed the need to distinguish two main chemical platforms for L. angustifolia: the first is a volatile essential oil rich in linalool and linalyl acetate; the second is a polar extract rich in phenolic acids and flavonoids. These fractions, obtained from the same raw material, differ in their biological targets and behavior in tests. The essential oil may be superior in antimicrobial and central nervous system effects, while the hydroethanolic extract may be superior in antioxidant and anti-inflammatory tests. However, this hypothesis needs to be confirmed by the chemical profile and dose-response relationship for each batch. The sharp differences in the amount of components in the literature are a methodological problem. For example, Özderin identified protocatechuic acid, 3,4-dihydroxybenzaldehyde and vanillic acid as important phenolics [4], while other studies report high levels of rosmarinic acid or flavonoids [2, 5]. This discrepancy is explained by the morphological composition of the sample, geographical origin, solvent polarity and analytical standards. Therefore, the results should be reported not by the general name "lavender extract", but through the chain "which organ - which solvent - which chemical profile - which dose".

In the proposed study design, three independent batches of raw materials are first chemically profiled; then the IC_{50}/MIC values of the total extract and active fractions are determined; finally, the activity is attributed to individual or synergistic components by biodirected fractionation. Statistical analysis is performed to check for normal distribution, using ANOVA for groups and appropriate post-hoc tests, and nonlinear regression for dose–response. Results should be reported as mean ± standard deviation and 95% confidence intervals.

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This approach not only reveals the presence of activity, but also its reproducibility and chemical basis.

CONCLUSION

Lavandula angustifolia provides two important sources of biologically active substances: an essential oil rich in terpenes and a polar extract rich in phenolic compounds. It is advisable to separate the essential oil by hydro- or microwave hydrodistillation, and the phenolics by ethanol-water ultrasonic extraction. GC–MS/GC–FID reliably characterizes volatile markers, while HPLC–DAD or UPLC–ESI–MS/MS reliably characterizes the phenolic profile. In pharmacological evaluation, antioxidant, antimicrobial, anti-inflammatory, and neuropharmacological tests should be performed in conjunction with chemical standardization, positive/negative controls, dose–response, and cytotoxicity data. In the future, it is important to study the phenophase and agroclimatic chemotypes of L. angustifolia varieties grown in Uzbekistan, identify active components through biodirected fractionation, and evaluate safety indicators.

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