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COMPARATIVE EVALUATION OF ANTIMICROBIAL POTENTIAL OF *ACHILLEA FILIPENDULINA*, *PRANGOS FERULACEA*, AND *PELARGONIUM GRAVEOLENS* ESSENTIAL OILS

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Essential oils (EO) extracted from *Achillea filipendulina*, *Prangos ferulacea*, and *Pelargonium graveolens* were investigated for their extraction yields and antimicrobial activity. Aerial parts of the plants were harvested during the flowering or early flowering stages from different regions of Armenia and subjected to hydrodistillation using a Clevenger-type apparatus. The yield of essential oils reached 0.5% for *A. Filipendulina*, approximately 4% for *P. Ferulacea* and 0.1–0.2% for *P. graveolens.* Antimicrobial activity was evaluated using disk-diffusion assays against a panel of Gram-positive and Gram-negative bacteria, including antibiotic-resistant *Escherichia coli* strains, as well as yeast species and presented by their minimum inhibitory concentration values (MIC). All tested EOs exhibited bactericidal activity with effective MIC values, although their efficacy was strain dependent. Among the investigated oils, *P. graveolens* EO showed the broadest antimicrobial spectrum, demonstrating strong antibacterial activity and exclusive anti-yeast effects. Growth kinetics analysis further confirmed the inhibitory impact of the EOs on both antibiotic-sensitive and resistant *E. coli* strains, as evidenced by reduced specific growth rates and prolonged generation times. Overall, these findings indicate that the studied essential oils, particularly *P. graveolens*, represent promising natural antimicrobial agents and warrant further investigation.

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Keywords**:** plant secondary metabolites, antibiotic activity, MIC, growth kinetics.

Introduction. The Armenian flora supports a rich tradition of medicinal and aromatic plant use [1]. Three species were selected with both ethnobotanical significance and modern pharmacological interest are *Achillea filipendulina* (Asteraceae), *Prangos ferulacea* (Apiaceae) and *Pelargonium graveolens* (Geraniaceae) for the investigation. Although taxonomically distinct, these plants converge in yielding volatile oils and polyphenolic constituents that underpin antimicrobial, antioxidant and anti-inflammatory activities [2–4].

According to some investigations the Achillea genus plants are chemically diverse; *A. filipendulina* in particular produces a complex mixture of volatile terpenoids and non-volatile phenolics. The essential oil (EO) fraction is typically dominated by monoterpenes and sesquiterpenes (for example, 1,8-cineole, camphor, borneol, and various germacrenes or chamazulene precursors depending on

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chemotype), while the non-volatile fraction contains flavonoids (apigenin, luteolin glycosides), phenolic acids (chlorogenic and caffeic acids) and tannins. Alkaloids are generally low or absent (https://doi.org/10.1080/10412905.2021.1885510). The relative proportions vary with genotype, phenological stage and environmental factors (soil, altitude, harvest time). The chemical composition determines the biological activity of the plant extracts or EO [5–7].

The investigations of Achillea species report a broad range of activities that can be largely attributed to the synergy of volatiles and phenolics. Antimicrobial effects against Gram-positive and Gram-negative bacteria, and certain fungal species, are commonly demonstrated and associated with EO terpenoids and phenolic compounds. Anti-inflammatory and analgesic activities correlate with flavonoid and sesquiterpene content, while antioxidant capacity is mainly linked to phenolic acids and flavonoids [8].

Additional properties reported in related Achillea taxa – wound-healing, spasmolytic and hepatoprotective activities – make *A. filipendulina* an interesting candidate for further bioactivity screening and phytochemical standardization [9].

In Armenia, Achillea species occur naturally in steppe, meadow and across low to montane elevations (https://ace.aua.am/wp-content/uploads/2020/05/Expanding_Plant_Species.pdf).

The *P. ferulacea* (Apiaceae family), produce an essential oil rich in monoterpenes (often α- and β-pinene, p-cymene, limonene and related hydrocarbons) and oxygenated terpenoids depending on chemotype [3]. Importantly, Prangos species are well known for coumarins and furanocoumarins, which contribute to biological activities but also demand careful toxicological consideration (phototoxicity) [10].

The investigations on *P. ferulacea* and other species report antimicrobial and antifungal activities attributable to EOs; antioxidant effects are linked to polyphenols; and cytotoxic or enzyme-modulating activities are often traced to coumarin derivatives [11].

P. ferulacea is native to the Caucasus Region and typically occupies calcareous slopes, open rocky places and montane meadows. In Armenia it is most likely encountered in foothill to subalpine zones, where soil is well drained and often limestone-derived [12].

P. graveolens (geranium) is primarily valued for its EO – geranium oil, which is rich in oxygenated monoterpenes. The oil commonly contains high proportions of citronellol and geraniol, with ancillary amounts of linalool, citronellyl formate, isomenthone, and various esters and aldehydes. Non-volatile constituents include tannins and small amounts of flavonoids. The precise oil profile varies widely with cultivar and growth conditions; commercial geranium oil chemotypes are often selected for elevated citronellol/geraniol ratios [13].

Geranium oil exhibits antimicrobial, anti-inflammatory, and insect-repellent properties. Citronellol and geraniol contribute to broad-spectrum antibacterial and antifungal actions, while antioxidant activity is moderate and typically associated with minor phenolic components. Topical preparations can show wound-healing and anti-inflammatory effects; systemic uses require careful formulation to avoid skin irritation [14].

The aim of the present research was to investigate some features of antibiotic activity of the EO extracted from *A. filipendulina*, *P. ferulacea* and *P. graveolens* against different Gram-positive and Gram-negative bacteria as well as some yeasts.

Materials and Methods.

Plant Material Collection and Essential Oil Extraction. *Achillea filipendulina* Lam. plants were harvested from the Vayots Dzor Province of Armenia (Noravank gorge) at 1770 *m* above sea level, in May–June, during the early flowering period.

Prangos ferulacea L. Lindl plants were harvested from the territory of Yerevan (Armenia) at 850–1000 *m* above sea level, in May–June, during the early flowering period.

Pelagonium graveolens L'Hér plants were harvested from Armavir Province of Armenia (v. Nor Kesaria) at 900–950 *m* above sea level, in May, during the flowering period.

EO has been extracted from the freshly collected aerial parts of the plant *via* the traditional hydro-distillation method. The oil was extracted using Clevenger-type apparatus as described before % [15].

Disk-diffusion Antimicrobial Activity Assay, Investigated Strains and Growth Conditions. The antimicrobial activity of *A. filipendulina*, *P. ferulacea* and *P. graveolens* EO was evaluated using the agar disk-diffusion method [5, 15]. Sterile cellulose disks (6 *mm* in diameter) were impregnated with EO concentrations ranging from 3.4 to 100 $\mu g/mL$, diluted in 96% ethanol. Ethanol served also as the negative control, kanamycin (50 $\mu g/mL$) was also used as a negative control to assess resistance, while tetracycline (15 $\mu g/mL$) was served as a positive control. The inhibition zone diameters were measured after 24 *h* of incubation at 37°C. For these cells from one colony were transferred to the liquid medium and grown overnight at 37°C. An overnight culture obtained after 24 *h* was inoculated in MP liquid media (peptone – 20 g/L, glucose – 2 g/L, NaCl – 5 g/L, K_2HPO4 – 2 g/L, pH 7.5). For the agar disk-diffusion method the media was supplemented with agar (17 g/L). The test panel included both Gram-negative and Gram-positive bacterial strains (*Escherichia coli* K-12, kanamycin-resistant *E. coli* pARG-25 (carry a high-copy-cloning plasmid, which has a KanR cassette, providing resistance to kanamycin), ampicillin-resistant *E. coli* DH5α-pUC18 (carry a low-copy-cloning pUC18 plasmid, providing resistance to ampicillin), *Bacillus subtilis* WT-A17, *Staphylococcus aureus* WDC 5233) as well as yeast species (*Debaryomyces hansenii* WDC M104 and *Candida guilliermondii* NP-4). The microbial strains were purchased from the Depository center of Scientific and Production Center "Armbiotechnology" of the National Academy of Sciences of Armenia. Antimicrobial efficacy was expressed in terms of minimal inhibitory concentration (MIC) values.

Determination of Bacterial Growth Parameters. The generation succeeding factor (G), the specific growth rate (μ) of bacteria, and the cell doubling time were determined by bacterial growth in liquid media, and measuring the density of the solution by using the DEN-1 McFarland Densitometer ("Biosan", Latvia). The μ was calculated by the following formula:

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1},$$

where $\ln N_2 - \ln N_1$ – logarithmic difference of doubled optical reading, $t_2 - t_1$ – difference between doubling time. The cell doubling time was determined by the $\ln 2/\mu$ equation [16].

Chemicals and also and reagents were obtained from “Sigma-Aldrich Co. Ltd.” (Taufkirchen, Germany), “Carl Roth GmbH & Co. KG” (Karlsruhe, Germany), and “VWR International” (Pennsylvania, USA).

Experimental data represent the mean ± standard deviation (SD) from three biologically independent replicates.

Results and Discussion. According to our results, the average yield of the *A. filipendulina* essential oils was 0.5%, meanwhile the same parameter for the *P. ferulaceae* was calculated to be around 4%. Scientific literature state that these results exceeded the any previously documented data for these plant species [17, 18]. For instance, Bagherifar et al. (2019) documented the data of 10 Prangos species, where the maximal yield of EO for immature seed and leaf samples were 3.0 ± 0.16% and 0.79 ± 0.03%, respectively [18]. Literature suggests also that *A. filipendulina* EO yields vary by plant part and developmental stage, with reported values ranging from 0.11% for stems to 0.77% for leaves, and 0.22% to 0.78% for total aerial parts during different growth phases [19] and average yield was 0.1–0.2%.

The optimal yield can be influenced by environmental factors and collection time, with 100% flowering often showing higher yields and favorable components for essential oil production.

Antibacterial Activity. The increased multidrug resistance of bacterial strains led to the increased severity of diseases caused by them. Moreover, the ability of bacteria to form biofilm-associated drug resistance has further increased the bacterial infections [20]. In addition, usage of antibacterial agents at higher doses may cause toxicity in humans. In this regard, plant extracts and EOs are potential candidates as antimicrobial agents [15, 20]. The present investigation revealed that the most potent EO is one extracted from *P. graveolens*, against which all tested microbial species expressed the rather high sensitivity in disc-diffusion test (see Table).

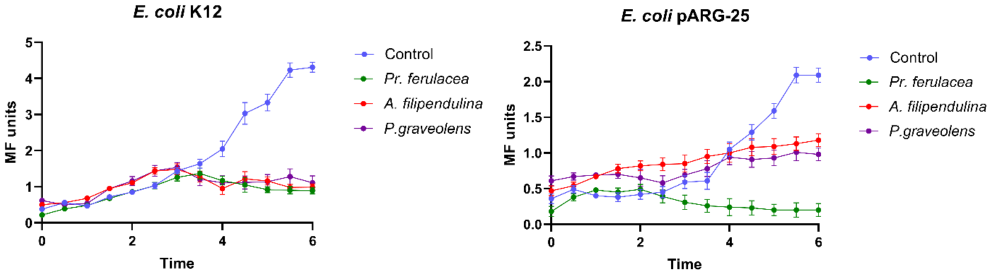
The MIC values of A. filipendulina, P. graveolens, and P. ferulacea EOs against the selected microbial strains

	A. filipendulina	*P. graveolens*	*P. ferulacea*
Bacterial strains	MIC values, $\mu g \cdot mL^{-1}$		
E. coli K-12	25	25	25
E. coli pARG-25	25	25	25
E. coli DH5α-pUC18	100	25	25
S. aureus WDC5233	25	100	–
B. subtilis WT-A17	25	25	50
C. guilliermondii NP-4	–	25	–
Deb. hansenii WDC M104	50	25	–

Overall, all three EOs exhibited comparable activity against *E. coli* K-12 and *E. coli* pARG-25 with MIC values of 25 $\mu g \cdot mL^{-1}$. However, against *E. coli* DH5α-pUC18, *A. filipendulina* EO showed reduced efficacy (MIC = 100 $\mu g \cdot mL^{-1}$), while *P. graveolens* and *P. ferulacea* EOs maintained higher inhibitory activity at 25 $\mu g\cdot mL^{-1}$, suggesting possible plasmid-related resistance mechanisms affecting susceptibility. Among Gram-positive bacteria, *A. filipendulina* EO demonstrated suppressing activity against *S. aureus* (MIC = 25 $\mu g\cdot mL^{-1}$) (Fig. 1), whereas *P. graveolens* EO was less effective (MIC = 100 $\mu g\cdot mL^{-1}$) and *P. ferulacea* EO showed no detectable activity. In contrast, both *A. filipendulina* and *P. graveolens* EOs were equally effective against *B. subtilis* (MIC = 25 $\mu g\cdot mL^{-1}$). Notably, *P. graveolens* EO was the only extract exhibiting anti-yeast activity, effectively inhibiting *C. guilliermondii* and *D. hansenii* with MIC values of 25 $\mu g\cdot mL^{-1}$, whereas *A. filipendulina* EO showed weaker activity against *D. hansenii* (MIC = 50 $\mu g\cdot mL^{-1}$). Collectively, these findings suggest that *P. graveolens* EO possesses the broadest antimicrobial spectrum, including antifungal effects.

Fig. 1. The influence of different concentrations of *A. filipendulina* EO on *S. aureus* WDC5233.

The MIC values determined are acceptable as effective, and the action of EO in this study was evaluated as bactericidal.



a b

Fig. 2. The growth curves of *E. coli* K-12 (a) and kanamycin resistant *E. coli* pARG-25 (b) under the treatment with *A. filipendulina, P. graveolens,* and *P. ferulacea* EOs. Control is the growing curve of untreated bacteria.

The further investigation of EOs were carried out using their MIC values. According to our results, the investigated EOs expressed highly suppressive influence on two the specific growth kinetics of two selected bacterial strains – *E. coli* K-12 and *E. coli* pARG-25 (Fig. 2). The selection of this bacterial strains based on out own results, according which all three EOs expresses the equal influence on them (see Table).

The values of calculated growth rate constants (μ) and generation time (G) were also calculated for *E. coli* K-12 and *E. coli* pARG-25 treated with EOs ($t_0 = 0$ and $t = 1$ h). The values of μ for untreated control cells as well as treated with *P. ferulacea*, *A. filipendulina*, and *P. graveolens* EOs were 0.472, 0.456, 0.42, and 0.39 h^{-1}, respectively, for *E. coli* K-12. The mean generation time G for the same variants were 1.47. 1.52, 1.65, and 1.78 h, respectively. For the kanamycin-resistant *E. coli* pARG-25 cells in the same conditions the μ values were 0.65, 0.434, 0.452, 0.198 h^{-1} for untreated control and treated with *P. ferulacea*, *A. filipendulina*, and *P. graveolens* EOs, respectively. G values were 1.06638, 1.597113, 1.533511, 3.500743 h, respectively.

Conclusion. The presented study demonstrates that the essential oils of *A. filipendulina*, *P. graveolens*, and *P. ferulacea* exhibit notable antimicrobial activity together with comparatively high extraction yields. All tested essential oils showed bactericidal effects with effective MIC values, although their activity was strain dependent. Among them, *P. graveolens* essential oil displayed the broadest antimicrobial spectrum, including pronounced antibacterial and anti-yeast activity. Growth kinetics analyses further confirmed the inhibitory effects of the essential oils on both antibiotic-sensitive and resistant *E. coli* strains. Overall, these findings highlight the potential of the investigated essential oils, particularly *P. graveolens*, as promising natural antimicrobial agents warranting further study.

This study was done in Department of Biochemistry, Microbiology and Biotechnology and Research Institute of Biology, Yerevan State University. The plant samples are available in the Department of Restoration and Conservation, Institute of Ancient Manuscripts, Matenadaran, Yerevan, Armenia.

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Լ. Յու. ՄԱՐԳԱՐՅԱՆ

ACHILLEA FILIPENDULINA, *PRANGOS FERULACEA* ԵՎ *PELARGONIUM GRAVEOLENS* ԲՈՒՅՍԵՐԻ ԵԹԵՐԱՅՈՒՂԵՐԻ ՀԱԿԱՄԱՆՐԷԱՅԻՆ ՆԵՐՈՒԺԻ ՀԱՄԵՄԱՏԱԿԱՆ ԳՆԱՀԱՏՈՒՄ

Achillea filipendulina, *Prangos ferulacea* և *Pelargonium graveolens* բույսերից արդյունահանված եթերայուղերը ուսումնասիրվել են դրանց հակամանրէային ակտիվության դրսևորման տեսանկյունից: Բույսերի վերգետնյա մասերը հավաքվել են ծաղկման շրջանում Հայաստանի տարբեր շրջաններից և ենթարկվել հիդրոդիստիլյացման՝ օգտագործելով Քլևենջեր տիպի սարք:

Ստացված եթերայուղերի արտադրողականությունը *A. filipendulina*-ի համար հասել է 0,5%-ի, *P. ferulacea*-ի համար՝ մոտավորապես 4%-ի, *P. graveolens*-ի՝ 0,1–0,2%-ի: Հակամանրէային ակտիվությունը գնահատվել է սկավառակային դիֆուզիոն մեթոդով՝ գրամ-դրական և գրամ-բացասական մանրէների մի շարք խմբերի նկատմամբ, ներառյալ հակաբիոտիկ-դիմացկուն *E. coli* շտամների, ինչպես նաև որոշ խմորասնկերի և արդյունքները ներկայացվել են դրանց նվազագույն արգելակող կոնցենտրացիայի արժեքներով (ՆԱԿ): Բոլոր փորձարկված եթերայուղերը ցուցաբերել են հակամանրէային ակտիվություն՝ իսկ դրանց արդյունավետությունը կախված էր մանրէի շտամից: Ուսումնասիրված եթերայուղերի շարքում *P. graveolens*-ի եթերայուղը ցուցաբերել է ամենալայն հակամանրէային սպեկտրը՝ ցուցաբերելով ուժեղ հակաբակտերիալ և հակախմորասնկային ակտիվություն: Աճի կինետիկայի վերլուծությունը հետագայում հաստատել է եթերայուղերի արգելակող ազդեցությունը ինչպես *E. coli* հակաբիոտիկ-զգայուն, այնպես էլ՝ կայուն շտամների վրա, ինչի մասին են վկայում աճի տեսակարար արագության նվազումը և կրկնապատկման ժամանակը: Ընդհանուր առմամբ, այս արդյունքները ցույց են տալիս, որ ուսումնասիրված եթերայուղերը, մասնավորապես *P. graveolens*-ը, ներկայացնում են խոստումնալից բնական հակամանրէային նյութեր և պահանջում են հետագա հետազոտություն:

Л. Ю. МАРГАРЯН

СРАВНИТЕЛЬНАЯ ОЦЕНКА АНТИМИКРОБНОГО ПОТЕНЦИАЛА ЭФИРНЫХ МАСЕЛ *ACHILLEA FILIPENDULINA, PRANGOS FERULACEA* И *PELARGONIUM GRAVEOLENS*

Были исследованы эфирные масла (ЭМ), извлеченные из *Achillea filipendulina, Prangos ferulacea* и *Pelargonium graveolens*, на предмет их антимикробной активности. Надземные части растений собирали в период цветения в различных регионах Армении и подвергали гидродистилляции с использованием аппарата типа Клевенджера. Выход эфирных масел достигал 0,5% для *A. Filipendulina*, приблизительно 4% для *P. Ferulacea* и 0,1–0,2% для *P. graveolens*. Антимикробную активность оценивали с помощью диск-диффузионного анализа против ряда грамположительных и грамотрицательных бактерий, включая устойчивые к антибиотикам штаммы *Escherichia coli*, а также виды дрожжей, и представляли значения минимальной ингибирующей концентрации (МИК). Все протестированные эфирные масла проявляли бактерицидную активность с эффективными значениями МИК, хотя их эффективность зависела от штамма. Среди исследованных масел эфирное масло *P. graveolens* показало самый широкий антимикробный спектр, продемонстрировав сильную антибактериальную активность и противодрожжевое действие. Анализ кинетики роста дополнительно подтвердил ингибирующее действие эфирных масел как на чувствительные, так и на устойчивые к антибиотикам штаммы *E. coli*, о чем свидетельствует снижение удельной скорости роста и увеличение времени генерации. В целом, эти результаты показывают, что изученные эфирные масла, особенно *P. graveolens*, являются перспективными природными противомикробными средствами и заслуживают дальнейшего изучения.