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И73 **Интродукция, сохранение и использование биологического разнообразия флоры** : материалы международной научной конференции, посвященной 90-летию Центрального ботанического сада Национальной академии наук Беларуси (Минск, 28 июня – 1 июля 2022 г.). В 2 ч. Ч. 1 / Нац. акад. наук Беларуси [и др.]. ; редкол.: В.В. Титок [и др.] – Минск : Белтаможсервис, 2022. – 526 с.

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RESEARCH ON THE DOMESTICATION OF IMPORTED ESSENTIAL OILS AND MEDICINAL PLANTS FROM BELARUS INTO VIETNAM

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Summary. This Report presents the results of the Project «Research on importing a selected number of potential medicinal plants and essential oil plants into Vietnam and Belarus» No. QTBY01.05/19-20, which was conducted under the Bilaterial Cooperation of the Vietnam Academy of Science and Technology (VAST).

ИССЛЕДОВАНИЯ ПО ИНТРОДУКЦИИ ПРЯНО-АРОМАТИЧЕСКИХ РАСТЕНИЙ ИЗ БЕЛАРУСИ ВО ВЬЕТНАМ

Луу Дам Н.А., Буи В.Х., Нгуен Ч.М., Нинх Х.Ю. Кухарева Л., Попов Е., Титок В.

Резюме. В данной статье представлены краткие результаты проекта по изучению пряно-ароматических и лекарственных растений во Вьетнаме и Беларуси, который проводился в рамках двустороннего сотрудничества Вьетнамской академии наук и технологий и Национальной академии наук Беларуси.

Introduction. The project has fulfilled its main goals, which were: (1) to research the transformation mechanism of imported plant varieties in different ecological habitats, and (2) Adding new essential oils and medicinal species to the plant collections of Vietnam and Belarus.

The research results have opened new prospects to domesticate many imported essential oil and medicinal plants of economic value from temperate countries to solve the current and urgent demand for developing medicinal plants.

The two parties also exchanged several medicinal plant varieties with good yield and quality in each country, which in turn has added new varieties of essential oils and medicinal plants to the collections of Vietnam and Belarus.

During the period 2018–2019, the Central Botanical Garden side has exchanged over 10 valuable medicinal plant varieties and essential oils for Vietnam. After two years of research and evaluation of species exchanged from the Central Botanical Garden of Belarus, among which two medicinal plant varieties were assessed to be well adapted to the conditions of migration; and were evaluated for their biological activity: Peppermint – *Mentha piperita* and Common sage: *Salvia officinalis.*

Evaluation of antifungal and antimicrobial of essential oils

Antimicrobial and Antifungal Assay

Antimicrobial and antifungal assays were carried out using *E. coli* (ATCC25922), *P. aeruginosa* (ATCC27853), *S. enterica* (ATCC12228), *E. faecalis* (ATCC13124), *S. aureus* (ATCC25923), *B. cereus* (ATCC13245), and *C. albicans* (ATCC1023). Stock solutions of samples were prepared in DMSO, and the assays were carried out in 96-well microtiter plates against the microbial strains (5×10^5 CFU/mL) using a modification of the published method (Andrews 2001). After incubation for 24 hours at 37 °C, the absorbance at 650 nm was measured using a microplate reader (BioRad, USA). Minimum inhibitory concentrations (MIC) were detected as the minimum concentration at which at least 50 % of microbial and fungal growth was missing. Streptomycin and cycloheximide were used as reference compounds.

Determination of antimicrobial activity by agar well diffusion method

Culture media: LB (Luria Bertani Broth) for bacterial strains: *B. subtilis, S. aureus, E. coli, P. aeruginosa, P. mirabilis, M. luteus, P. vulgaris and S. typhi;* Hansen for the fungus *C. albicans.*

After the microbial strains were activated from the stock tube on concentrated LB or Hansen medium, one colony was transferred to 5 ml of liquid LB or Hansen medium and shaken overnight at 37 ˚C (for bacteria) and 30 ºC (for fungi). Cultivate and restore microorganisms in a suitable liquid medium for 2–4 hours at 37 ºC (for bacteria) and 30 ºC (for fungi) until the microbial fluid reaches OD_{600} = 0 ,5–0.6, equivalent to a microbial density of 4-5 x 10^8 cells/ml. Inoculate 100 µl of the microbiological solution onto a petri dish containing 15 ml of solid medium suitable for each type of microorganism. Then, punch wells with a diameter of 90 mm.

Carry out sample dilution: the initial sample is diluted in Dimethyl Sulfoxide (DMSO) to a series of 3 concentrations of 10 % (100 mg/ml), 20% (200 mg/ml) and 30 % (300 mg/ml respectively)./ml). Aspirate 50 µl of the test sample into the wells on a petri dish, the negative control is 50 µl DMSO, the positive control is 50 µl Gentamicin 200 µg/ml (for bacteria) and nystatin 100 µg/ml (for fungi). The test dishes were cultured in an incubator at 37 ˚C for 16 hours (for bacteria) and at 30 ˚C for 48 hours (for fungi).

Bacterial inhibitory activity was assessed by measuring the microbial inhibitory ring diameter (DK) according to the formula: DK (mm) = D–d, wherein D = the sterile ring diameter, and d = agar well diameter. 0 represents the sample with no antibacterial activity.

Primary results

Obtained essential oil content at 2.2 % proportionate to the freshness

Peppermint – *Mentha piperita* flowers in the June 2019 crop in Hanoi

Fig. 1. The trial garden of domesticated plant varieties in Hanoi and Lam Dong Province (the Central Highlands), Photo: LD. Ngoc Anh

Chemical analyses of the essential oil of Peppermint *M. piperita* were analysed and 40 components were identified, accounting for 100 %. Among them, the composition of substances accounting for a high proportion were: Menthol (19.31 %), 3-Terpinolenone (5.66 %); Piperitone (11.05 %), Pulegone (12.42 %), Limone (6.1 %), and 1.8-Cineole (4.37 %). The essential oil obtained from the leaves was used for bioactivity evaluation (table 1).

Table 1. **Antimicrobial activity results of peppermint essential oil grown in Vietnam**

TT	Sample	Species name						
		Grams (+)			Grams (-)			Yeast
		E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginsa*	*S. enterica*	*C. albicans*
MIC (µg/ml)								
first	*M. piperita*	64	128	64	128	–	128	128
Antibiotic	Streptomycin	256	256	128	32	256	128	–
	Cyclohexamide							32
IC 50 (µg/ml)								
	M. *piperita*	34.22	65.33	32.22	67.22	–	64.55	65.67

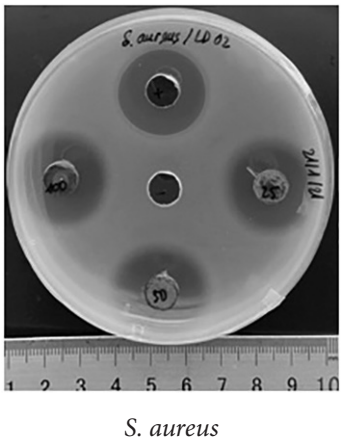
Results showed that Peppermint essential oil had strong resistance to five strains of bacteria including: *E. faecalis, S. aureus, B. cereus, E. coli,* and *S. enterica,* and one yeast strain *C. albicans.*

Salvia L. is the largest genus in the *Lamicaceae* with nearly 1000 species across the world. It is a medicinal plant containing essential oils. *Salvia* L. is used to treat over 60 diseases ranging from pain to epilepsy, mainly colds, bronchitis, bleeding and menstrual disorders. Seeds grown after harvest were dried in the sun until dry, then stored in aluminum-coated bags for preservation before planting. The tree flowered and grew well in the climatic conditions of the Central Highlands (Tay Nguyen – Vietnam). In Hanoi, after 2 trial seasons, flowering season were not recorded.

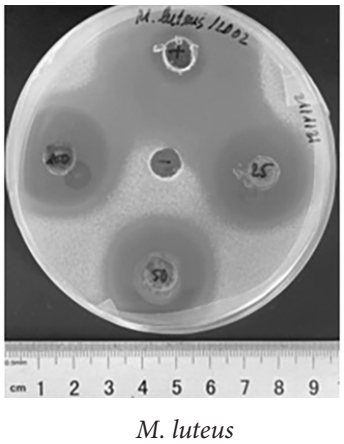
When investigating the antibacterial ability of the twig and leaf extracts of *Salvia officinalis* grown in the Central Highlands, the results showed that the extract was resistant to *M. luteus, S. aureus,* and *C. albicans* fungi (table 2, fig. 2).

Table 2. **Diameter of Antibacterial zone of essential oil of Salvia officinalis against tested microbial strains**

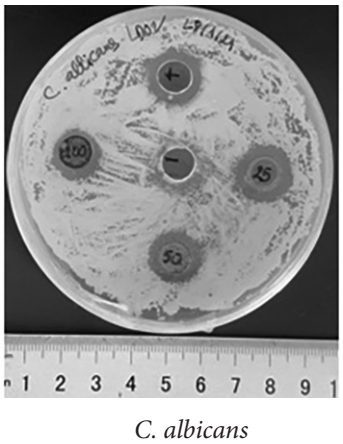
Microbial strains	Antibacterial zone (mm)			
	C. albicans	*M. luteus*	*S. aureus*	*P. vulgaris*
LD02 - *Salvia*				
25 µg/ml	6.97±0.25	19.16±0.15	14.02±0.25	0
50 µg /ml	5.83±0.28	19.3±0.57	16.06±0.2	0
100 µg /ml	5±0.2	19.2±0.26	17.1±0.26	0
Nystanin (100 µg g/ml)	4.07±0.2	–	–	–
Gentamicin (200 µg g/ml)	–	5.03±0.2	15.53±0.28	5.06±0.4



S. aureus
+: Gentamicin 200 g/ml
-: DMSO



M. luteus
+: Gentamicin 200 g/ml
-: DMSO



C. albicans
+: Nystatin 100 g/ml
-: DMSO

Fig. 2. Diameter of Antibacterial zone of essential oil of Salvia officinalis against tested microbial strains, *Photo: Nguyen Chi Mai*

The promising results from the primary studies of two medicinal plant varieties containing essential oils showed that Vietnam has suitable conditions to grow essential oil and medicinal plants of temperate origin (fig. 3).

Fig. 3. Model of Imported essential oils and medicinal plants from Belarus to Central Highlands – Vietnam, January 2019, Photo: LD. Ngoc Anh

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