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CHEMICAL COMPOSITION OF THE GARDEN NASTURTIUM ESSENTIAL OIL AND ANTIBACTERIAL ACTIVITY OF FRESH JUICE OF THE HERB

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Abstract

Medicines from plants are widely used in the complex treatment of many diseases. Therefore, theoretical and practical interest is the in-depth study of the herb of garden nasturtium. This plant has a long history of usage. Nasturtium is used as an antiscorbutic, general tonic, hemocathartic, antimicrobial, vitamin, anti-cold, and antisclerotic agent. It contains polyphenols, flavonoids, alkaloids, tannins, carbohydrates, terpenoids, carotenoids, mucus, salts of potassium, phosphorus, iodine, iron, phytoncides, macro-and micronutrients, starch, sugar, resins, pectins, phytosterols, B vitamins, myrosin enzyme. Nasturtium is rarely used in scientific medicine. The study aimed to research the essential oil of fresh herbs and to establish the antibacterial activity of nasturtium herb juice. The components of essential oil present in the herb were studied by GC/MS analysis. The results of the analysis showed that the garden nasturtium herb has thirty components of essential oil, of which twenty-three were identified. Benzyl isothiocyanate (34.04%), heptacozan (15.09%), benzyl alcohol (13.01%), nonacosan (9.28%), and pentacosane (5.02%) dominated among the identified components. Antibacterial and antifungal properties of the juice of garden nasturtium herb were determined by the "wells" method. The juice of garden nasturtium was very active against *Staphylococcus aureus* ATCC 6538 ((27±0.96) mm) and active against *Candida albicans* ATCC 885-653 ((20±1.64) mm), *Pseudomonas aeruginosa* ATCC 9027 ((18±0.9) mm), *Escherichia* coli ATCC 25922 ((17±0.9) mm).

Keywords: garden nasturtium, herb, essential oil, juice, antibacterial activity, benzyl isothiocyanate, benzyl alcohol

Introduction

The widespread use of antibacterial drugs not only did not live up to expectations, but also created a number of new problems that need to be addressed immediately. These are, first of all, extremely high rates of growth and spread of resistance of infectious agents to antimicrobial drugs, frequent side effects on the human body, the appearance of allergic reactions [1-4]. Another, no less frequent consequence of the use of antibiotics is a violation of the intestinal microflora – dysbiosis. It is accompanied by increased reproduction of non-resident microflora, which are mainly opportunistic gram-negative microorganisms and fungi of the genus *Candida* [5].

The attention of researchers is attracted not only by antibiotics of microbial origin, but also by antimicrobial substances obtained from other sources, one of which are plants [6, 7]. Phytotherapy has a number of edges, such as it is low-toxic, has a mild pharmacological effect and high tolerability, regardless of the age of patients [8, 9]. The appearance of synthetic drugs, which mainly imitate the biologically active substances of plants, has not reduced the role of natural drugs [10]. Experimental studies and clinical use of herbal medicines show their great value in the treatment of many, especially chronic diseases [11]. Antimicrobial substances of plants, although often inferior to antibiotics of microbial origin in activity in vitro, but have a number of properties that provide them with advantages in the treatment of certain diseases caused or complicated by microbial flora. In particular, the presence of a complex of active substances with a wide range of pharmacological activity in plants and the fact that they are in a certain ratio, their high bioavailability, contributes to the optimal impact on the human body and effective treatment of diseases. That is, in contrast to traditional antibacterial drugs, most antibiotics of plant origin, in addition to antimicrobial action, cause a pronounced positive effect on the functioning of physiological systems of the macroorganism [6, 7]. In addition, the use of antimicrobial drugs of plants in chemotherapy of some infectious processes, especially local, limits the irrational use of classical antibiotics. This prevents the development of a number of side effects, including slowing down the selection of antibiotic-resistant bacterial strains. Thus, plants are a promising source for antimicrobials. However, only a small number of plants are used in official medicine. The remaining species are used only in folk medicine, because they are insufficiently studied pharmacologically. Such plants also include garden nasturtium (*Tropaeolum majus* L.).

Nasturtium herb, especially due to the contained essential oils, has a detrimental effect on pathogenic microorganisms and stimulates the body's protective functions, as well as improves metabolic processes. Therefore, German herbalists often use it to treat and prevent influenza. They also recommend nasturtium for anemia and arerosclerosis.

Butnariu M., Bostan C. (2011) studied the anti-inflammatory and antimicrobial activity of individual chemical compounds of nasturtium essential oil, which was isolated from leaves and flowers. The isolated components of essential oils were identified by color reactions and gas chromatography with a mass spectrometric detector [12].

The herb, leaves, flowers, buds, fruits and seeds of nasturtium are used as a diuretic and anti-inflammatory agent, as well as for scurvy, anemia, bronchitis, kidney stones, metabolic disorders. Decoction of the herb is recommended as a vasodilator in coronary heart disease, anemia, bronchitis, pneumonia and asthma. The juice helps with inflammation of the urinary tract, burns, lipomas, polyps, skin diseases, alcohol tincture of a mixture of nasturtium and nettle leaves – for alopecia, baldness, herbal infusion stimulates hair growth and relieves dandruff [13]. It has been experimentally proven that 2.5–10 % infusion of nasturtium underground organs significantly increases diuresis in experimental rats and excretion of Na + ions. Nasturtium compounds that cause a diuretic effect have been shown to be non-toxic [14-16], and the mechanism involves a system of prostaglandins.

Barboza et al. investigated the effect of long-term administration of nasturtium extract to rats on calcium excretion and its content in bone tissue. It is proved that nasturtium extract has a pronounced diuretic effect with prolonged administration, does not affect the excretion of calcium and potassium, reduces lipid levels [17].

In 1954, Winter noted the antibiotic properties of common nasturtium. Good results in the treatment of urogenital infections and inflammatory diseases of the respiratory tract were also described. Weiss (1974) successfully used common nasturtium in the intervals between intensive antibiotic treatment for pyelonephritis [18].

Analysis of available scientific literature sources has shown that common nasturtium is a valuable ornamental, medicinal and food plant. It contains many important biologically active substances, has been used in folk medicine both in Ukraine and in other countries, as an anti-inflammatory, antimicrobial, vitamin, sedative, anti-cold and antisclerotic agent. Nasturtium is rarely used in scientific medicine. Phytochemical and pharmacological studies of the fresh aboveground part of the plant were practically not performed. The aim of our research was to study the essential oil of fresh herb and to establish the antibacterial activity of nasturtium herb juice.

Methods

The objects of the study were the fresh herb and juice of garden nasturtium (*Tropaeolum majus* L.) The aboveground part of garden nasturtium was collected during a flowering period in the experimental plots of I. Horbachevsky Ternopil National Medical University (Druzhba village, Terebovlya district, Ternopil region). The raw material was authenticated by prof. Svitlana Marchyshyn [19, 20]. A voucher specimen is kept at the Department of Pharmacognosy and Medical Botany, TNMU [21, 22].

Microorganisms

An experiment used a standardized daily suspension of testing strains of the following microorganisms: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *and Candida albicans* ATCC 885-653 [23]. Cell concentration was 0.5 McFarland (used to compare the standard turbidity).

GC/MS analysis of the essential oil

GC/MS analysis of the essential oil was performed using gas chromatograph Agilent 6890N with 5973inert mass detector (Agilent Technologies, USA) [24, 25]. Samples were analyzed on a silica capillary column HP-5MS length – 30 m, internal diameter – 0.25 mm, the diameter of sorbent grain – 0.25 µm [26]. Injections were made in the split mode 1:50. First, the temperature was set at 50°C, and then at a rate of 3°C/min was raised to 220°C. Helium was used as the carrier gas at a constant flow rate of 1.0 ml/min. The sample with a volume of 2 µl was injected in a splitless mode. The mass spectrometer recorded the entire spectrum (SCAN mode) in range from 38 to 400 m/z, using electronic ionization energy at 70 eV [27, 28]. Scan rate (electronic) was 10 000 amu/s.

Identification of components of the essential oil was based on their retention times compared to standards and mass spectral library NIST 02 [29]. Quantitative content was calculated in relation to the area of the peaks of the components to the sum of the areas of all peaks on the chromatogram.

Antibacterial and antifungal test

The antimicrobial action of fresh juice of the herb was studied *in vitro* [30-32]. This activity was determined directly after the preparation of the study samples. Antibacterial action on microorganisms was studied by two-fold serial dilutions in a liquid nutrient (meat infusion broth) and agar diffusion ("wells" method) [33]. The cultural properties of bacterial isolates were studied by inoculation on conventional and selective media.

Standardization of the agar diffusion was secured by 6 mm "well" diameter and 10 mm medium thickness. Around the cylinders, the upper layer was poured, consisting of nutrient agar medium, melted and cooled to 40 °C, in which the appropriate standard daily culture of the test microbe was introduced. Preliminarily, the upper layer was mixed well to form a homogeneous mass. After infusion of testing strains into a nutrient, "wells" were filled with droplets of herb homogenate and tincture diluted in meat infusion broth. The Petri dishes were dried for 30-40 min at room temperature. Then there were placed into the thermostat and incubated at 37 °C. Results were evaluated in 24

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hours by measuring the diameter of the inhibition zone around a "well" [34].

Statistical analysis

Results were determined using Statistica v 10.0 (StatSoft I nc.) program. Results were represented as mean ± SEM [35]. Statistical significance of differences between mean values was assessed by the Student's t-test. The difference between the values was considered reliable if the likelihood was *p<0.05 [35-39].

Results and Discussion

The results of a gas chromato-mass spectrometric study of the component composition of the essential oil of garden nasturtium fresh herb are presented in Figure 1.

As a result of the conducted studies, thirty components of essential oil were detected in the garden nasturtium fresh herb, of which twenty-three were identified (Table 1). Their content was 86.77 % of the total amount of all components of essential oils. Benzyl isothiocyanate, heptacozan, benzyl alcohol, nonacosan, pentacosane dominated among the identified twenty-three components of essential oils, their content was 34.04%, 15.09%, 13.01%, 9.28%, and 5.02%, respectively.

Benzyl isothiocyanate is an isothiocyanate that exhibits anti-parasitic, antioxidative, immunomodulatory, antibiotic, anti-atherosclerotic, anti-metastatic, anti-angiogenic, anticancer chemotherapeutic and chemopreventive activities [40-46]. Isothiocyanates are natural antimicrobial compounds [47]. Also, benzyl isothiocyanate modulates the intracellular localization of the transcription factor Forkhead box O 1 (FOXO1). FoxO transcription factors can antagonize insulin effects and trigger a variety of cellular processes involved in tumor suppression, longevity, development, and metabolism [48]. Benzyl isothiocyanate inhibits the growth of *Trypanosoma* and displays antibacterial efficacy against *Campylobacter* by disrupting metabolic processes [40]. Benzyl alcohol is an aromatic alcohol. In the human body, benzyl alcohol is metabolized into benzoic acid, which reacts with glycine and is excreted as hippuric acid [49]. It is generally accepted that benzyl alcohol exerts antimicrobial action and the non-specific ability to alter membranes in Gram-negative bacteria [50-52].

Studies of antimicrobial and antifungal activity showed (Table 2) that juice of garden nasturtium herb has the lowest antibacterial activity against bacilli. *Bacillus subtilis* strain was insensitive and the growth retardation of microorganisms was (15±1.1) mm. The test culture of *Staphylococcus aureus* was highly sensitive to the juice of garden nasturtium herb. The zone of growth retardation around the "well" was (27±0.96) mm. Gram-negative rods turned out to be sensitive to the juice of garden nasturtium herb. However, the antibacterial properties of the sample against cultures of *Pseudomonas aeruginosa* and *Escherichia coli* were slightly lower than against Staphylococcus aureus. The juice inhibited the growth of test strains of *Escherichia coli* and *Pseudomonas aeruginosa* around the "well" by (17±0.9) mm and (18±0.9) mm, respectively. Yeast fungi were also sensitive to the sample. The growth of *Candida albicans* was delayed by (20±1.64) mm.

Conclusions

The essential oil present in the fresh herb of garden nasturtium was studied by GC/MS analysis. The results of the analysis showed that the fresh herb has thirty components of essential oil, of which twenty-three were identified. Benzyl isothiocyanate (34.04%), heptacozan (15.09%), benzyl alcohol (13.01%), nonacosan (9.28%), and pentacosane (5.02%) dominated among the identified components.

Antibacterial and antifungal properties of the juice of garden nasturtium herb were determined by the "wells" method. The juice of garden nasturtium was very active against *Staphylococcus aureus* ATCC 6538 and active against *Candida albicans* ATCC 885-653, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 25922.

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Figure 1. GC/MS chromatogram of essential oil of garden nasturtium herb (fresh)

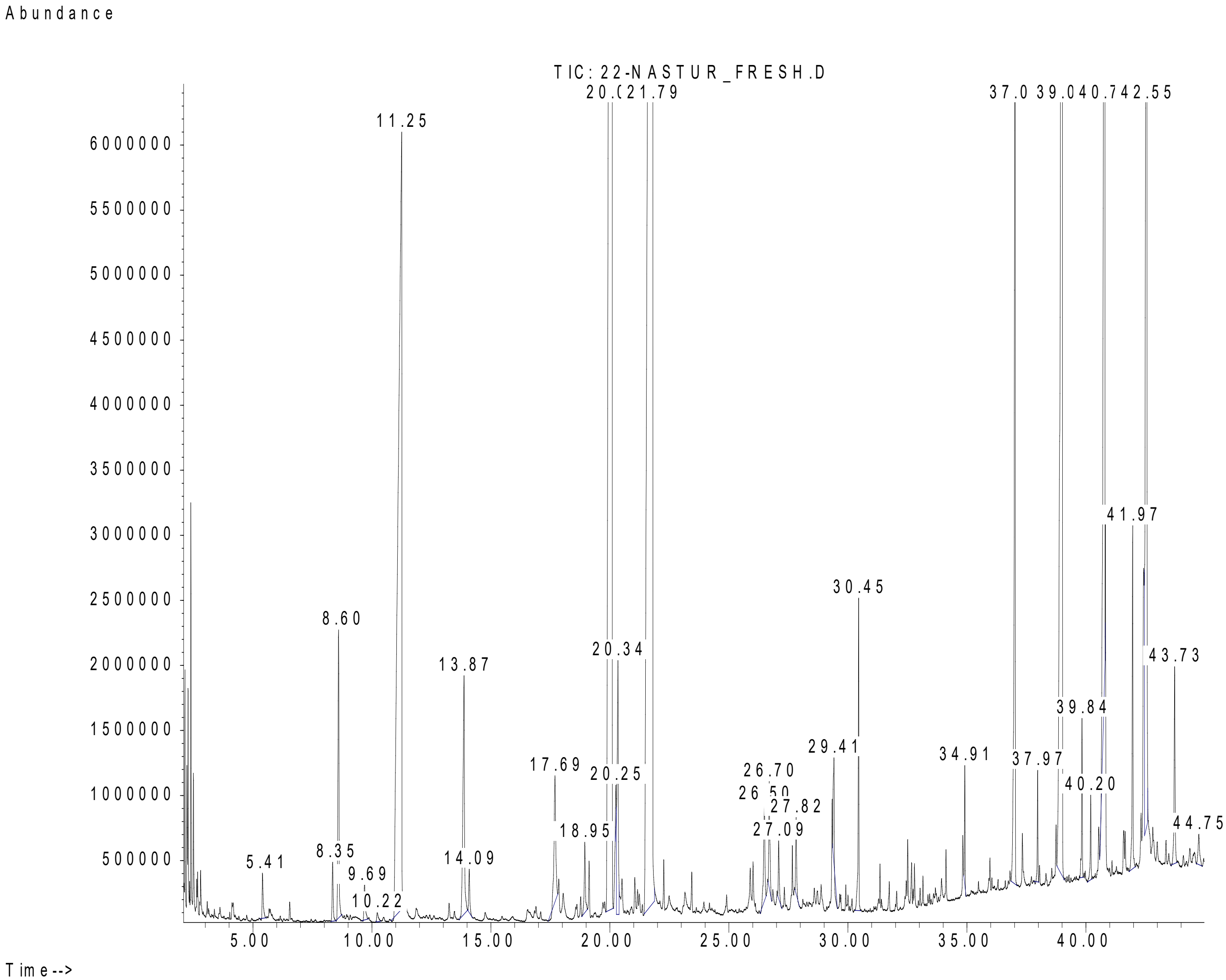

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Table 1. Component composition of the essential oil of garden nasturtium herb (fresh)

Components of essential oils	%
cis-3-Hexen-1-ol	0.22
trans-2-Heptenal	0.28
Benzaldehyde	1.44
cis-2,4-Heptadienal	0.22
trans-2,4-Heptadienal	0.08
Benzyl alcohol	**13.01**
beta-Phenylethyl alcohol	1.78
Benzyl isocyanate	0.23
2,3-Dihydrobenzofuran	1.01
not detected	0.39
cys-Cinnamyl alcohol	0.13
trans-Cinnamyl alcohol	1.54
Benzyl isothiocyanate	**34.04**
4-Methoxybenzyl isothiocyanate	0.61
3-oxo-α-ionol	0.55
epoxy-3-oxo-α-ionol	0.26
cis-Ethyl-p-methoxycinnamate	0.34
trans-Ethyl-p-oxycinnamate	0.43
not detected	1.14
Tricosane	0.32
Pentacosane	**5.02**
Hexacosane	0.34
Heptacozan	**15.09**
Octacosane	0.55
not detected	0.30
Nonacosan	**9.28**
not detected	1.28
not detected	8.98
not detected	0.93
not detected	0.21

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Table 2. Analysis of the antimicrobial and antifungal activity of the juice of garden nasturtium by "wells" method

Testing culture of microorganisms	The diameter of the growth retardation of microorganisms, mm
Bacillus subtilis ATCC 6633	15±1.1
Escherichia coli ATCC 25922	17±0.9
Staphylococcus aureus ATCC 6538	27±0.96
Pseudomonas aeruginosa ATCC 9027	18±0.9
Candida albicans ATCC 885-653	20±1.64

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