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INVESTIGATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE HERB OF *TROPAEOLUM MAJUS* L.

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Abstract

The medicinal properties of *Tropaeolum majus* L. herb have been known since ancient times and are the result of the availability of different groups of biologically active compounds. The components of essential oil present in the herb of *Tropaeolum majus* L. were studied by GC/MS analysis. The results of the analysis showed that the *Tropaeolum majus* L. herb has 53 components of essential oil, of which 35 (66.04% of the total amount of all components of essential oils) were identified. γ-sitosterol (7.84%), nonacosan (7.45%), stigmasterol (2.19%), and β-sitosterol (2.07%) dominated among the identified components. Antibacterial activity of herb homogenate and tincture of *Tropaeolum majus* L. was determined "wells" method. Tincture of *Tropaeolum majus* L. herb was determined very active against *Staphylococcus aureus* ATCC 6538 (26±2.1 mm) and active against *Escherichia coli* ATCC 25922 (23±1.4 mm), *Pseudomonas aeruginosa* ATCC 9027 (22±1.6 mm), *Candida albicans* ATCC 885-653 (17±2.03 mm). Herb homogenate of *Tropaeolum majus* L. was found active against *Staphylococcus aureus* ATCC 6538 (24±1.19 mm), *Escherichia coli* ATCC 25922 (22±1.8 mm), *Pseudomonas aeruginosa* ATCC 9027 (20±2.2 mm), *Candida albicans* ATCC 885-653 (16±2.3 mm), and nonactive against *Bacillus subtilis* ATCC 6633 (9±1.1 mm). To summarise it can be suggested that the *Tropaeolum majus* L. is a perspective plant for medicinal purposes because of its excellent role in many biological processes.

Keywords: *Tropaeolum majus* L., herb, essential oil, γ-sitosterol, stigmasterol, β-sitosterol, antibacterial activity

Introduction

Since prehistoric times, people have used plants to treat many diseases. Despite the significant progress of modern organic chemistry, pharmaceutical science, which has ensured the production of high-quality synthetic pharmacologically active drugs that have found use in pharmacy and medicine, the popularity of herbal remedies around the world is rapidly increasing [1, 2]. Therefore, today, modern medicine is increasingly using herbal remedies along with synthetic drugs, antibiotics. Phytotherapy has a number of advantages over traditional therapy with using oral synthetic agents, namely, it is low-toxic, has a mild pharmacological effect and possibility to be used for long periods of time without significant side effects, is well combined with synthetic drugs, has a complex activity through a number of biologically active compounds [3-7].

Today, a significant number of plants are used in official medicine of Ukraine and the world and more than a thousand species, which are recommended by folk medicine, are promising for phytotherapy and the pharmaceutical industry [8-10].

These are species of natural flora and cultivated, which are grown in specialized farms, botanical gardens, in backyards as medicinal plants. Many of them are ornamental. The vast majority of these plants have medicinal properties and are important for the prevention and treatment of many diseases [11-13]. Such plants include the garden nasturtium *Tropaeolum majus* L. of the family *Tropaeolaceae.* It is a herbaceous plant native to South America. In Ukraine, it has long been grown as ornamental. The plant has healing properties; it is used as an antiscorbutic, general tonic, hemocathartic, antimicrobial, vitamin, anti-cold and antisclerotic agent [14].

All parts of the plant contain isosulfan glycoside glycotropeolin (about 0.1%). When the structure of the raw material is disturbed, glycotropelin is converted into benzyl isothiocyanate by fermentation, which is the main component of the plant's essential oil [15]. Four fractions of essential oil in garden nasturtium were established. One of them, isolated by vacuum distillation, was pharmacologically active. It is called tropeolin. Tropeolin is a mobile light liquid with a sharp specific odor. In addition, nasturtium seeds contain a highly active but unstable antibiotic. In the aboveground part of the plant, there are alkaloids (0.11%), tannins, carbohydrates (3-4%), mucus, salts of potassium, phosphorus, iodine, iron, phytoncides, macro-and micronutrients, starch, sugar, resins, pectins, phytosterols, B vitamins, vitamin D, myrosin enzyme. Also, nasturtium contains polyphenols and low molecular weight phenols, such as chlorogenic acid, as well as flavonoids (isoquercitrin, quercetin, kaempferol). The flowers of the plant contain carotenoids and anthocyanin dyes (pelargonidin), and the seeds contain isoanthocyanins [16, 17]. A significant amount of isoquercitrin and kaempferol glycosides was detected by HPLC in nasturtium leaves. Phytochemical studies by American scientists have shown that nasturtium leaves also contain terpenoids [18, 19]. Bazylko et al have found the presence and quantitative content of quercetin-3-O-glucoside (isoquercitrin) and kaempferol-3-O-glucoside (astragalin) in aqueous extracts of nasturtium herb [20]. In addition, these scientists have analyzed ethanol and aqueous extracts from the leaves and flowers of nasturtium and found a much higher quantitative content of flavonoid compounds [21]. However, the analysis of available scientific literature sources indicates a lack of information about the qualitative composition and quantitative content of essential oil of nasturtium, as well as the lack of studies of antibacterial activity of homogenates of herbs and tinctures, so this study is relevant.

Methods

The object of the study was the *Tropaeolum majus* L. herb. This raw material was harvested during a mass flowering period (August - September) in the experimental plots of I. Horbachevsky Ternopil National Medical University (Druzhba village, Terebovlya district, Ternopil region). The test raw material was authenticated by prof. Svitlana Marchyshyn (I. Horbachevsky TNMU, Ternopil, Ukraine) [22, 23]. A voucher specimen of *Tropaeolum majus* L. is kept at the Department of Pharmacognosy and Medical Botany, TNMU [24-26]. The study raw material was dried using the conventional method and stored in paper bags in a dry, protected from direct sunlight place [27, 28].

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 [29]. 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 of *Tropaeolum majus* L. herb was performed using gas chromatograph Agilent Technology 6890N with mass detector 5973 inert (Agilent Technologies, USA) [30-33]. 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 [34-36]. Injections were made in the split mode 1:50 [37]. 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 [38, 39]. The sample with a volume of 2 µl was injected in a splitless mode. Detection was held in the SCAN mode in the range of (38–400 m/z) [40-43].

Identification of components of the essential oil was based on comparing their retention times with retention times of standards of the mass spectral library NIST 02 [44-47]. 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 the herb homogenate and tincture was studied *in vitro* [48-50]. This activity was determined directly after the preparation of the study samples. Antibacterial and antifungal action on microorganisms was studied by two-fold serial dilutions in a liquid nutrient (meat infusion broth) and agar diffusion ("wells" method) [51]. 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 hours by measuring the diameter of the inhibition zone around a "well" [52].

Statistical analysis

Results were determined using Statistica v 10.0 (StatSoft I nc.) program. Results were represented as mean ± SEM [53-55]. 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 [56-61].

Results and Discussion

The results of a gas chromato-mass spectrometric study of the component composition of the essential oil of *Tropaeolum majus* L. herb are presented in Figure 1.

As a result of the conducted studies, 53 components of essential oil were detected in the *Tropaeolum majus* L. herb, of which 35 were identified (Table 1). Their content was 66.04% of the total amount of all components of essential oils. γ-sitosterol, nonacosan, stigmasterol, and β-sitosterol dominated among the identified thirty-five components of essential oils, their content was 7.84%, 7.45%, 2.19%, and 2.07%, respectively. γ-sitosterol has been to possess an antihyperglycemic property by rising insulin secretion in reply to glucose verified with immune histochemical research of pancreas [62-64]. Also, Sundarraj et al. was established the ethnomedical use of γ-sitosterol against cancer through the growth inhibition and cell cycle detention on the apoptosis of cancer cells, which showed that γ-sitosterol was cytotoxic against liver and colon cancer cell lines and that this

effect was mediated by downregulation of c-myc expression and induction of the apoptotic pathways [65, 66]. β-sitosterol is the most common phytosterol. Its epimer is γ-sitosterol [62]. Stigmasterol (Wulzen anti-stiffness factor or stigmasterin) is an unsaturated sterol that has been isolated from medicinal plants. Stigmasterol is utilized in many chemical processes which are designed to yield numerous semi-synthetic and synthetic compounds for the pharmaceutical industry. It acts as an intermediate in the biosynthesis of estrogens, corticoids, androgens, in the synthesis of vitamin D_3, and acts as a precursor in the synthesis of progesterone [67, 68]. Stigmasterol is an important constituent and has been isolated from plants [69].

Studies of antimicrobial and antifungal activity showed (Table 2) that nasturtium tincture has the lowest antibacterial activity against bacilli. *Bacillus subtilis* strain was insensitive and the growth retardation of microorganisms was (14±1.1) mm. In general, the insensitive strain of *Bacillus subtilis* was to the herb homogenate. Against the strain of *Staphylococcus aureus* tincture and homogenate of nasturtium herb acted most effectively. The test culture of *Staphylococcus aureus* was highly sensitive to the tincture and sensitive to the herb homogenate. Zones of growth retardation around the "well" ranged from (24±1.19) mm (herb homogenate) to (26±2.1) mm (tincture). Gram-negative rods turned out to be sensitive to raw materials. However, the antibacterial properties of all samples against cultures of *Escherichia coli* and *Pseudomonas aeruginosa* were slightly lower than against *Staphylococcus aureus*, the raw material inhibited the growth of test strains of *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027 around the "well" by (17±0.9) – (23±1.4) mm. Yeast fungi were also sensitive to these samples. The growth of *Candida albicans* ATCC 885-653 was delayed by (16±2.3) – (20±1.64) mm.

Conclusions

The components of essential oil present in the herb of *Tropaeolum majus* L. were studied by GC/MS analysis. The results of the analysis showed that the herb has 53 components of essential oil, of which 35 were identified. γ-sitosterol (7.84% of the total amount of all components of essential oils), nonacosan (7.45%), stigmasterol (2.19%), and β-sitosterol (2.07%) dominated among the identified components.

Antibacterial activity of herb homogenate and tincture of *Tropaeolum majus* L. was determined "wells" method. Tincture of *Tropaeolum majus* L. herb was determined very active against *Staphylococcus aureus* ATCC 6538 and active against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 885-653. Herb homogenate of *Tropaeolum majus* L. was found active against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 885-653, and nonactive against *Bacillus subtilis* ATCC 6633.

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Figure 1. GC/MS chromatogram of essential oil of *Tropaeolum majus* L. herb

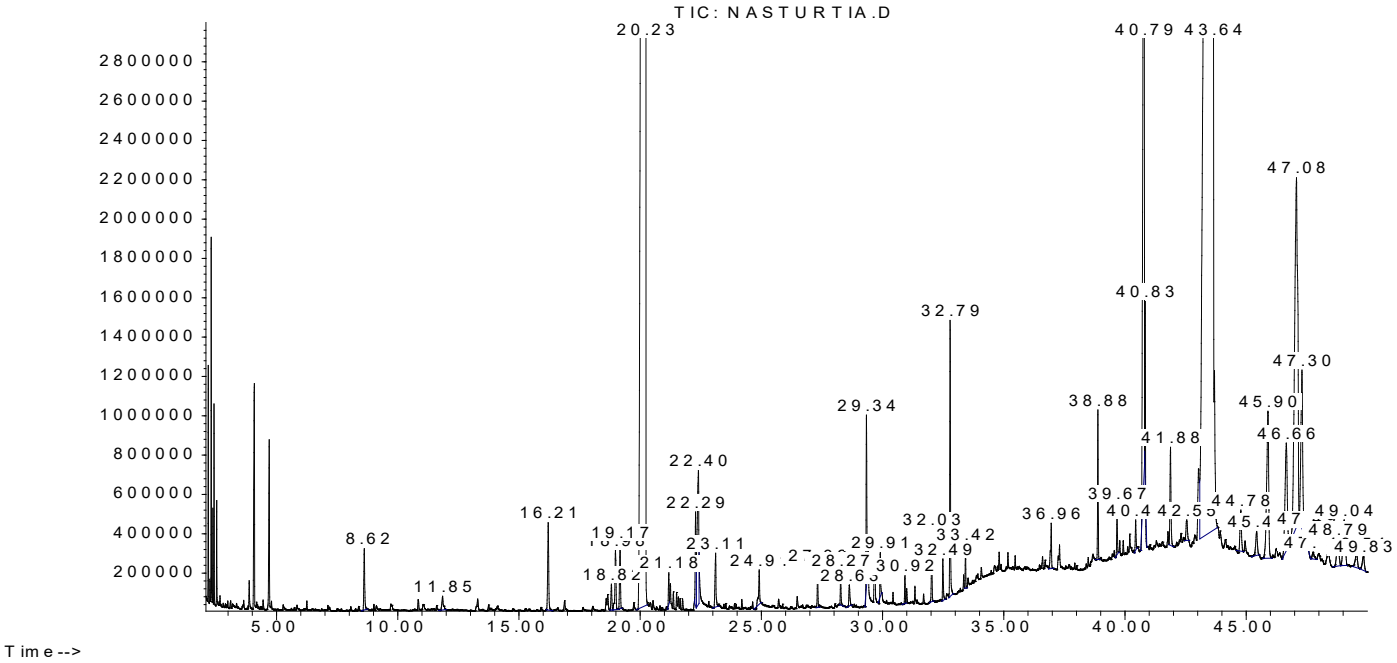

Table 1. Component composition of the essential oil of *Tropaeolum majus* L. herb

Components of essential oils	%
Benzaldehyde	0.40
2,4-Heptadienal	0.04
Decan	0.03
Limonene	0.06
Benzyl alcohol	0.06
not detected	0.14
Benzonitrile	0.02
Undecane	0.09
Benzyl isocyanate	0.04
Neomenthol	0.03
Menthol	0.67
Dodecan	0.06
3-Ethyl-4-methyl-1H-pyrrole-2,5-dione	0.04
Benzyl isothiocyanate	0.06
Tetradecane	0.26
2-Benzylacetamide	1.61

not detected	0.19
N-Benzylacetamide	0.39
Dihydroactinidiolide	0.24
not detected	0.04
not detected	0.08
not detected	0.22
N-Benzylidenebenzylamine	0.18
Loliolid	0.16
cis-Neophytadiene	1.05
Hexahydrofarnesyl acetone	0.35
cis-trans-Neophytadiene	0.13
trans-Neophytadiene	0.19
not detected	0.37
not detected	0.24
Phytol	1.52
Ethyl linoleate	0.18
Pentacozan	0.33
Heptacozan	0.68
13-Docosenamide	0.24
not detected	0.18
nonacosan	**7.45**
not detected	0.54
not detected	0.75
not detected	0.21
not detected	0.41
not detected	0.29
stigmasterol	**2.19**
not detected	1.74
γ-sitosterol	**7.84**
β-sitosterol	**2.07**
not detected	0.21
not detected	0.10
Cycloartenol	0.44
Lupeol	0.75

α-Tocopherol acetate	0.23
Sitostenon	0.21

Table 2. Analysis of antibacterial activity of the tincture and homogenate of *Tropaeolum majus* L. herb by "wells" method

Testing culture of microorganisms	**The diameter of the growth retardation of microorganisms, mm**	
	Herb homogenate	**Tincture**
Bacillus subtilis ATCC 6633	9±1.1	14±1.1
Escherichia coli ATCC 25922	22±1.8	23±1.4
Staphylococcus aureus ATCC 6538	24±1.19	26±2.1
Pseudomonas aeruginosa ATCC 9027	20±2.2	22±1.6
Candida albicans ATCC 885-653	16±2.3	17±2.03