Citrus essential oils as biologically active substances in the human health preventive care

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Abstract. The work is devoted to the comparative analysis of the component composition of the essential oil of some species of the genus Citrus of different origin. Essential oil was extracted from the peel of kumquat *(Citrus japonica* Thunb.), lime *(Citrus aurantifolia (Christm.)* Swingle*),* and limequat (*Citrus x floridana* (J.W. Ingram & H.E. Moore) Mabb.*)* fruits by steam distillation using a modified Ginzberg receiver. The component composition of the essential oil has been established by the GC-MS method. In the studied species of the genus Citrus, the content of the main component of limonene varies from 50 to 95%. Differences are observed in the quantitative ratio of some components that relate to terpene hydrocarbons, alcohols, and their esters.

1 Introduction

The fruits of the Rutaceae family have been known and used in phytotherapy and medicinal purposes for more than three thousand years. The region of origin of citrus fruits is considered to be Indochina and Southern China [1]. From the XIV-XV century citrus fruits began to spread all over the world. Thus, kumquat, a fruit belonging to the Rutaceae family, appeared in Europe in the XIX century.

Rutaceae are a family of flowering plants, including woody shrubs and trees, as well as several grassy perennial plants. It consists of 160 genus and about 2070 species and is distributed worldwide, especially in moderate warm, and tropical regions. Kumquat fruits are small tart orange fruits that grow in East Asia. They are grown for the sake of edible pulp and sweet fleshy skin [2].

Lime is a citrus hybrid (C. hystrix×C. medica) native to tropical Southeast Asia. Lime is usually harvested while it is still green, its skin is thinner and it is appreciated for its distinctive taste [3].

The systematics of citrus fruits is quite complex. At the beginning of the last century, the oval kumquat was assigned to the subgenus Eufortunella F. Spp. Genomic analysis confirmed the relationship of kumquat to the Rutaceae family [4], commonly known as "citrus", although this refers to the genus of plants, not the family. Most of them are trees or shrubs, but there are also grasses. Some of the plants, especially representatives of the genus Citrus, are important food crops, and some are grown as garden ornamentals. [5].

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Many representatives of the citrus genus are known for their antimicrobial, antibacterial, and antioxidant activity [6, 7]. Plants contain many active compounds, such as alkaloids, tannins, volatile oils, resins, phenols and flavonoids, which are deposited in their specific parts, such as leaves, flowers, bark, seeds, fruits, roots, etc. [8] Their ability to inhibit the growth of pathogenic fungi of plants, the growth of tumor human cells, wound healing effect. Plants are a source of new chemical compounds that can potentially be used in medicine and for other purposes. The beneficial medicinal effects of herbal raw materials are usually the result of a combination of these products.

Plants are able to synthesize and release phytoncides into the environment - biologically active substances that neutralize pathogenic microorganisms, contributing to effective air purification. Essential oils of the citrus genus are widely used as flavoring ingredients in the food and alcoholic beverages, cosmetic and perfume industries [9]. It was found that extracts of various parts, essential oil of fruits and some pure compounds have biological activity, for example, repellent, antimicrobial, antioxidant and antitumor properties [10,11].

In traditional medicine, citrus fruits were considered an effective means of combating moths on clothes, as well as a mouthwash. In Western medicine, essential oil has been widely used as an antiseptic against bacteria, fungi, protozoa and insects due to the high content of phenolic compounds in them. Citrus plants have been widely used in the West to relieve scurvy caused by a significant decrease in vitamin C levels and as a symptomatic treatment for colds [12].

Moreover, some doctors have suggested that lemon juice counteracts obesity by lowering cholesterol and controlling insulin [13, 14].

Kumquat essential oil is obtained from small citrus fruits of the Fortunella Japonica tree. It has a bright, beautiful aroma and has many advantages: it contains various compounds, including terpenoid hydrocarbons, alcohols, ketones, esters, and aldehydes. The specific percentage of these compounds may vary. The antioxidant properties of this oil are widely known, the aroma of kumquat essential oil lifts the mood and tones up. Studies have been conducted on the effect of kumquat essential oil on cancer cells [15, 16].

Citrus fruits are distinguished by a wide variety of component composition of essential oils [17]. Nevertheless, studies have shown that the main component is linalool, and the other groups of compounds vary in their ratio and are responsible for the characteristic of the relationship to a particular species and, accordingly, a specific flavor. Linalool refers to two enantiomers of natural terpene alcohol found in many flowers and spicy plants. Linalool has many commercial uses, most of which are based on its pleasant floral aroma, with a hint of spice [18]. Its smell is similar to floral, spicy-woody, somewhat reminiscent of French lavender, bergamot oil or lily of the valley. Linalool is used in the production of soaps, fragrances, food additives, household goods, and insecticides [19, 20].

Despite the multilateral study of the biological activity of a large number of components of the essential oil of Citrus plants, some individual substances still remain not fully identified.

2 Materials and methods

To conduct a comparative analysis study, the fruits of kumquat (Citrus japonica Thunb.) originating from South Africa, lime (Citrus aurantiifolia (Christm.) Swingle.) originating from Mexico, kumquat (Citrus japonica Thunb.) [4], and limequat (Citrus x floridana (J.W. Ingram & H.E.Moore) Mabb.) were purchased on retail sale and grown in the greenhouse of the RSAU-MAA named after K.A. Timiryazev.

By steam distillation using a modified Ginzberg receiver [5], essential oil was extracted from the peel of fresh fruits. The duration of extraction is 10 hours. The layer of essential

oil cured from the receiver was weighed on microanalytical scales. The yield of essential oil was calculated for 100 grams of fresh fruit peel.

The analysis of the essential oil was carried out by the FSAEI HE "Russian State Agrarian University - Moscow Timiryazev Agricultural Academy" in the Educational and Scientific Center for collective use – "Service laboratory of complex analysis of chemical compounds". The solution of essential oil in hexane was analyzed by chromatomass spectrometry on the analytical complex "Clarus 600M" by Perkin Elmer (GC capillary column "Elite Wax" - 60 m x 0.32 mm x 0.5 μm; carrier gas helium - 1 ml/min, sample volume 0.5 ml, flow division 1/50; temperature mode: 60°C – 5 minutes, 3°/min to 195°C, isotherm 15 minutes, detector MS. MS mode: E+70eV, interface temperature – 210°C, source temperature – 180°C) [6].

The components of the essential oil were determined according to the data of a mass spectrometric detector with the processing of the mass spectra of all compounds by the search engine "NIST/ERA/NIH, ver.2-2020", and the final results from the RI library developed earlier at the Department of Organic Chemistry of the K.A. Timiryazev of the Russian State Agrarian University - Moscow Agricultural Academy based on the method of ellipsoidal distribution of n-alkanes in in the mode of arbitrary programming of the analysis temperature.

3 Results and discussion

The conducted studies have shown that the highest yield of essential oil was recorded in a limequat sample grown in a TAA greenhouse. The yield per 100 grams of fresh raw materials was 0.6%. In the peel of lime fruits from Mexico, the content of essential oil turned out to be two times less – 0.28%. The kumquat fruits grown in the TAA greenhouse contain 0.38% essential oil, and the fruits originating from South Africa contain 0.46% per 100 grams of peel.

As a result of the GC-MS analysis, mono- and sesquiterpene hydrocarbons, alcohols and their esters, aldehydes, and ketones were found and identified in the composition of the studied essential oil samples, which are presented in the table. The confidence interval calculated from four samples for components with a high oil content is in the range of plus or minus 1-1.5%. For compounds with concentrations within 1.0-0.1% - plus or minus 0.02-0.2%, and at concentrations below 0.01%, it should be said that they are present in the sample within this concentration.

Table 1. Component composition of essential oils (%).

Component	Limequat TSA	Kumquat TSA	Lime Mexico	Kumquat South Africa
□-Pinene	0.43	0.22	0.91	0.37
□-Thujene	0.15	0.00	0.10	0.03
□-Pinene	0.58	0.00	8.61	0.01
Sabinene	0.11	0.06	1.03	0.08
□-Myrcene	1.16	1.50	1.01	1.90

□-Phellandrene	0.03	0.03	0.02	0.03
□-Terpinene	0.26	0.00	0.12	0.01
D-Limonene	79.48	94.84	50.20	95.35
□-Phellandrene	0.19	0.22	0.87	0.24
□-Terpinene	11.81	0.02	5.59	0.02
p-Cymene	0.08	0.00	4.72	0.00
Terpinolene	0.54	0.00	0.37	0.01
Octanal	0.01	0.00	0.04	
Nonanal	0.06	0.00	0.03	0.00
Limonene 1,2-epoxide	0.12		0.01	0.02
Citronellal	0.06	0.00	0.16	0.00
Decanal	0.02	0.02	0.10	0.02
Linalyl formate	0.72	0.21	1.39	0.01
4-Terpenyl acetate	0.02	0.00	0.03	0.01
□-Bergamotene	0.21	0.00	0.02	0.02
Terpinen-4-ol	0.31	0.02	1.52	0.02
□-Caryophyllene	0.13	0.00	0.24	0.01
□-Caryophyllene	0.01	0.00	0.07	0.09
Carveol	0.17	0.00	5.27	0.01
□-Terpineol	0.55	0.02	2.64	0.04
□-Tepinyl acetate	0.67	1.59	1.99	0.85
□-Bisabolene	0.39	0.00	0.73	
Citral	0.27	0.00	8.36	0.02
□-Gurjunene		0.22	0.12	0.14
Geranyl iso-valerate	0.10	0.34	0.52	0.21
Perillal	0.01	0.01	0.13	
Neryl iso-valerate	0.11	0.00	0.95	
Geranyl propionate	0.09	0.11	0.21	0.01
Linolyl iso-valerate	0.11	0.00	0.88	0.17

In all samples, the main component is limonene. The ratio of components in essential oil is an indicative element in the characteristics of the studied species. So both samples of kumquat contain 95% limonene. Lime from Mexico - 50% limonene; limequat is a hybrid

form of kumquat (key lime x kumquat) – 80%. The samples of lime and limequat are dominated by monoterpene hydrocarbons in total – 23.4% and 14.8%, respectively, for example, □□-pinene: 0.43, 0.91; □pinene: 0.58, 8.61; □fellandren: 0.19, 0.87; □cimene: 0.08, 4.72; □terpinene: 11.81, 5.59%, respectively, which have high antifungal and antimicrobial activity. Lime essential oil is distinguished by a higher content of alcohols and aldehydes in comparison with other samples. The content of all classes of components characterizing kumquat shows the stability of the species regardless of growing conditions. The entire pool of essential oil synthesis fell on limonene. The detected and identified classes of compounds, such as mono- and sesquiterpene hydrocarbons, alcohols and their esters, ketones, and aldehydes, are determined equally in both kumquat samples.

4 Conclusions

The peel of limequat fruits grown in a greenhouse contains the largest amount of essential oil among the studied samples – 0.6%. Evaluation of the results of the component composition analysis of the essential oil of the peel of kumquat fruits of different origin showed the stability of the genetic apparatus of this species, since the quantitative ratio of the main component and minor compounds with a percentage of less than 0.1% in the oil are determined almost identically. The conducted study of the component composition of essential oils showed the presence of a large spectrum of biologically active substances that positively affect the human body, which confirms the undoubted relevance of further research of the biochemical features of the citrus genus.

Acknowledgements

The article was prepared with support of the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement № 075-15-2022-317 date April 20. 2022 on providing a grant in the form of subsidies from the Federal budget of Russian Federation. The grant was provided for state support for the creation and development of a Word class Scientific Center “Agrotechnologies for the Future”.

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