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RHEOLOGICAL PROPERTIES OF MONOTERPENE COMPOUNDS

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Annotation. Monoterpenes are the most interesting substrates with biological and chemical properties. Nowadays monoterpens structure didn't clearly recognized by several modern methods. A number of representatives of the terpene series from relevant literature sources were considered to compare their chemical activity, predict obstacles to rheological detection in order to avoid key errors in analytical analyses such as electrophoresis. To determine several qualities of it monoterpene contanting structure was observed and investigated by spectrophotometer and capillar phoresis. In this study, a colorimetric indicator assay was developed for rapid determination of monoterpenes in sweet orange (*Citrus sinensis*) essential oils. Capillar electrophoresis results showed that the main components in citrus essential oil are citral, which elicited greater extents of color changes as compared to other monoterpenes (limonene, α-pinene, β-pinene and terpinolene). The colorimetric indicator is potentially useful for rapid assessment of monoterpene oxidation in citrus essential oil and other citrus-based products.
Keywords: monoterpenes, reological properties, thin-layer chromatography, citral.

МОНОТЕРПЕНДІ ҚОСЫЛЫСТАРДЫҢ РЕОЛОГИЯЛЫҚ ҚАСИЕТТЕРІ

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Аңдатпа. Монотерпендер - биологиялық және химиялық қасиеттері бар ең қызықты субстраттардың бірі. Қазіргі уақытта монотерпендердің құрылымы заманауи әдістердің көмегімен толық анықталмаған. Тиісті әдебиет көздерінен алынған терпендер сериясының бірқатар өкілдері олардың химиялық белсенділігін салыстыру, электрофорез сияқты аналитикалық талдаулардағы негізгі қателіктерді болдырмау үшін реологиялық анықтаудағы кедергілерді болжау үшін қарастырылды. Олардың бірқатар қасиеттерін анықтау мақсатында монотерпен құрамы бар құрылым спектрофотометр және капиллярлық электрофорез

әдістерімен зерттелді. Бұл зерттеуде тәтті апельсиннің (*Citrus sinensis*) эфир майларындағы монотерпендерді жедел анықтау үшін колориметриялық индикаторлық әдіс әзірленді. Капиллярлық электрофорез нәтижелері цитрус эфир майының негізгі компоненті цитраль екенін көрсетті, ол басқа монотерпендерге (лимонен, α-пинен, β-пинен және терпинолен) қарағанда түс өзгерісін көбірек тудырады. Колориметриялық индикатор цитрус эфир майларындағы және басқа цитрус негізіндегі өнімдердегі монотерпендердің тотығуын тез бағалау үшін қолдануға мүмкіндік береді.

Түйінді сөздер: монотерпендер, реологиялық қасиеттер, жұқа қабатты хроматография, цитраль.

РЕОЛОГИЧЕСКИЕ СВОЙСТВА МОНОТЕРПЕНОВЫХ СОЕДИНЕНИЙ

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Аннотация. Монотерпены представляют собой одни из наиболее интересных субстратов, обладающих биологическими и химическими свойствами. В настоящее время структура монотерпенов до конца не определена с помощью ряда современных методов. Был рассмотрен ряд представителей терпенового ряда из актуальных литературных источников для сравнения их химической активности, прогнозирования препятствия для реологического детектирования во избежания ключевых ошибок в аналитичеких анализах, таких как электрофорез. Для выявления их свойств была изучена структура, содержащая монотерпен, с использованием спектрофотометрии и капиллярного электрофореза. В данной работе был разработан подход для быстрого определения монотерпенов в эфирных маслах сладкого апельсина (*Citrus sinensis*). Результаты капиллярного электрофореза показали, что основным компонентом эфирного масла цитрусовых является цитраль, который вызывает более выраженные изменения окраски по сравнению с другими монотерпенами (лимонен, α-пинен, β-пинен и терпинолен). Колориметрический индикатор может быть использован для быстрого определения реологических свойств и степени окисления монотерпенов в эфирных маслах цитрусовых и других продуктах на основе цитрусовых.

Ключевые слова: монотерпены, реологические свойства, тонкослойная хроматография, цитраль.

Introduction. Terpenes are a group of unsaturated hydrocarbons assembled based on two or more isoprene units. The chemical structures of the common monoterpenes (C10H16) and sesquiterpenes. The predominant monoterpene in cannabis is myrcene, which imparts earthy/grassy fragrance and exhibits sedative effects for medicinal uses. Myrcene and farnesene are industrially available and can be obtained from the fermentation of biomass-derived sugars such as sugar cane or biosynthesis, which makes them suitable as a raw material for sustainable fine chemicals. Trans--β-farnesene from sugar cane fermentation is also used for the production of squalene (Masyita, 2022:100217). For another example, Squalane is an oil-like terpene highly desirable in the cosmetic industry for skincare formulations due to its emollient properties. It has also been used widely as a food supplement, and in pharmaceuticals. Emulsions of squalane with surfactants are added to vaccines to enhance the immune response. The demand for squalane has steadily increased in recent years and is predicted to continue increasing (Martinez-Botella,2023). Diterpenes is forskolin could been used as a fluorescent marker for membrane adenylyl cyclase in living enteric neurons in the guinea

pigileum. Forskolin demonstates the activity of cyclase -type reactions. Forscolin is a suitable neural marker for identifying various classes of neurons. This was proved by colocalization experiments with specific calcium-binding proteins. Sesquiterpenes are composed of three isoprene units and often contribute to the distinct aromas of plants and fungi. Several sesquiterpenes have been studied for their potential medicinal properties. For example, artemisinin, a sesquiterpene lactone derived from sweet wormwood plants, is a critical element in the treatment of malaria (Stankova, 2024:1306-1319).

Another monoterpene commonly found in cannabis is limonene, which has citrus aroma commonly used in food flavoring and perfumery applications. Other major monoterpene fractions present in cannabis products include α-pinene, β-pinene, camphene, *p*-cymene, ocimene, and terpinolene (Abdollani et al.,2020, Ibrahim et al., 2019). As one of the most investigated medicinal plants, cannabis (*Cannabis sativa*) continues to gain attention in the contemporary world due to its psychoactive property, medicinal benefits, and increasing legalization in recreational products (Booth&Bohlmann,2019:67-62), (Mahamad et al., 2020:337-346), (Marangoni, 2019:1-6).

Among the various components of cannabis, cannabinoids and terpenes are two most essential groups of compounds that contribute to its bioactive characteristics (Leghissa, 2017:398-415). Various analytical methods have been developed for characterizing cannabinoids in cannabis, including gas chromatography (GC) or liquid chromatography (TLC) coupled with mass spectrometry (MS), infrared spectroscopy (Risoluti, at al., 2020:1777-1782). In comparison, analytical protocols for terpene analysis in cannabis are relatively underexplored, where GC remains the primary approach due to terpene's high volatility (Watanabe, 1979:321-326). Besides monoterpenes, sesquiterpenes such as β-caryophyllene,myrcene, α-humulene, and selinene, are the second most abundant group of chemical constitutes in the volatile fraction of cannabis (Gulluni, 2018), (Novak&Franz), (Amirgaliev et al., 2012).

Citral components have unique properties of enhancing chemoprevention of cancers, and the improvement of menopausal syndromes, osteoporosis, endometriosis, prostatic hyperplasia. It could be obtain from ginger (Zingiber officinale Roscoe) has been used as a food, spice, supplement and flavoring agent and shows us hepatoprotective, anti-inflammatory activities (Kiyama, 2020:108486). Citral is using as emulsion against various gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*), gram-negative bacteria (*Escherichia coli*), and fungi (*Candida albicans*). Synthetic stategies control a microbial growth and enhanced immune respounce. All of citral formulation showed remarkable capability of encapsulating essential oil and increasing antimicrobial properties (Mokarizadeh, 2017).

Terpenes and terpenoids possess a wide range of biological activities including anticancer, antimicrobial (Stankova, 2022:1306-1319), anti-inflammatory, antioxidant, and antiallergic.Major bioactive terpen compounds shed the light on disease management questions (Masyita, 2022).

Experimental part.

Procedure

One milliliter of extracts of different concentrations (from 0.018 to 2.5 mg/mL) is mixed with 2.5 mL of 0.2 M phosphate buffer (pH = 7) and 2.5 mL of 1% potassium ferricyanide $K_3Fe(CN)_6$. The whole set is heated in a water bath at 50˚C for 20 min. The tubes are centrifuged at 3000 rom for 10 min.

Determination of Flavonoids

To 1 mL of AlCl3 solution (2% dissolved in methanol), 1 mL of each sample and standard (prepared in methanol) was added. Absorbance was read after 10 minutes of incubation against the prepared reagent blank (J.C.Bakar et al.). Flavonoid concentrations were deduced from the calibration curve range (M.Morshedi, et al.).

Evaluation of Antioxidant Power by FRAP

The ferric reducing antioxidant power (FRAP) of the synthesized compounds was determined according to the method of Benzie and Strain (1996) with slight modifications. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a 10:1:1 (v/v/v) ratio. An aliquot of 100 μL of the sample solution of citral (dissolved in methanol) was added to 3.0 mL of FRAP reagent

and incubated at 37°C for 30 min in the dark. A calibration curve was constructed using standard $FeSO_4 \cdot 7H_2O$ solutions (100-1000 μM), and the results were expressed as μmol Fe(II) equivalents per gram of compound (μmol Fe^{2+}/g). All determinations were performed in triplicate, and mean values ± standard deviations were reported.

Determination of Antioxidant Activity by Thin-Layer Chromatography

Thin-layer chromatography is performed on a glass plate coated with a thin layer of stationary phase. The stationary phase is silica, which is responsible for adsorption and partitioning. The compounds to be separated from the extract are dissolved in the eluent vapour, which carries them away, separating them into small-diameter spots. The result is diffuse round spots (L.Gallego et al.).

Results and Discussion.

Extraction Yields

Two isomers were separated in hexane-ethyl acetate (8:2, v/v) Fig 1 shows why polar compounds are more prevalent in roots and leaves, but less so in stem barks. Concerning acetone extracts, yields are respectively 21.43% for stem barks, 20.73% for roots, and 11.43% high. Explanations could be that stem barks contain more semi-polar compounds, followed by roots and leaves. As for the yields of hexane extracts, the values obtained are very low compared with the other values. They are 17.01% for leaves, 12.21% for roots and 4.03% for stem bark. This can be explained by the lack of apolar compounds in stem bark, and their minimal presence in the plant.

The presence of citral in the methanolic solution was confirmed by thin-layer chromatography (TLC). The analysis was carried out on silica gel 60 F_{254} plates (Merck). The mobile phase consisted of hexane-ethyl acetate (8:2, v/v).

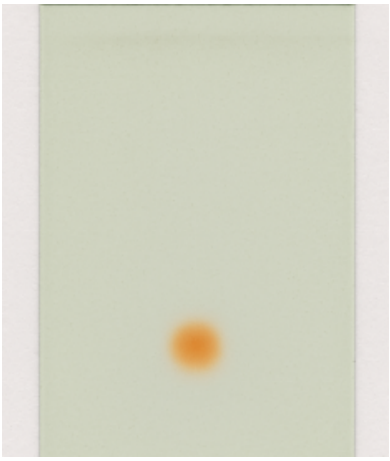

Fig. 1. Citral. The mobile phase consisted of hexane-ethyl acetate (8:2, v/v)

TLC of Citral

The appearance of an orange spot corresponding to citral was observed at an Rf value of approximately 0.45-0.50, which matched that of the authentic citral standard analyzed under the same conditions

Table 1. TLC of Citral (Rf = 0.45-0.50)

acetone	+
methanol	-
chloroform	+
hexane	+

The qualitative composition of the monoterpene fraction was confirmed by thin-layer chromatography on silica gel plates (SiO_2, 60 F_{254}, Merck). The mobile phase consisted of *n*-hexane-ethyl acetate (8:2, v/v). Visualization under UV light (254 nm) and subsequent treatment

with 2,4-dinitrophenylhydrazine reagent revealed characteristic orange-yellow spots corresponding to carbonyl-containing compounds. The main compound, identified as citral, exhibited an *Rf* value of 0.45-0.50, which was consistent with the authentic standard. The TLC profile demonstrated the presence of minor components corresponding to limonene and α-pinene, indicating partial oxidation of terpenoid structures during storage. These findings confirm the stability and purity of the isolated monoterpene fraction and demonstrate the suitability of TLC as a rapid screening method for terpene derivatives in essential oil systems.

Fig 2. illustrated the optical density of a molar solution. It is based on the chemical reaction of reduction of Fe3+ to Fe2+ present in the $K_3Fe(CN)_6$ complex. Significant indicator of a compound's potential antioxidant activity. The absorbance of the reaction medium is determined at a wavelength of 240-260 nm using a UV-Visible spectrophotometer.

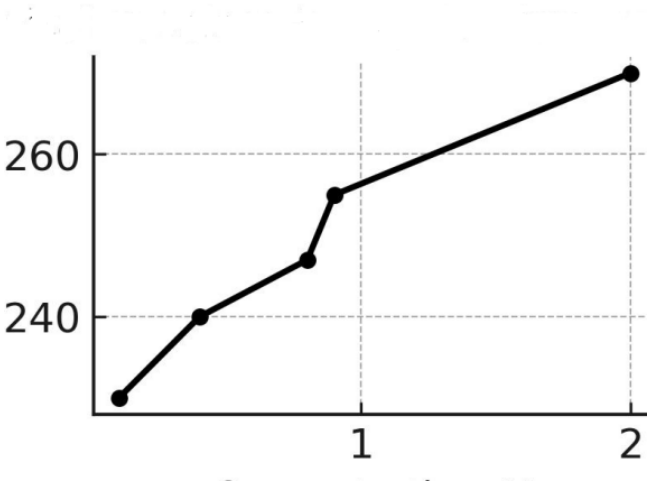

Fig. 2. Graph depending on the optical density of a molar solution

Phytochemical Screening is illustrated by EFG

The results of the phytochemical assays designed in Fig. 3. After development, the plates were air-dried and visualized under UV light at 254 and 365 nm, followed by spraying with citral reagent to detect the carbonyl group. Capillary electrophoresis allowed for a more detailed evaluation of the electrophoretic behavior of citral and related monoterpenes. Under optimized conditions (phosphate buffer, pH 7.0; applied voltage = 20 kV; capillary = 50 μm × 50 cm), well-resolved peaks were observed with retention times in the range of 2.8-5.4 min. The dominant signal corresponded to citral, confirming its major contribution to the essential oil composition. The obtained electropherogram exhibited a symmetrical peak shape and high reproducibility (RSD < 2%), reflecting both the homogeneity of the compound and the reliability of the analytical conditions. A comparative analysis of TLC and CE data showed excellent correlation between chromatographic mobility (*Rf*) and electrophoretic migration time, supporting the accuracy of compound identification.

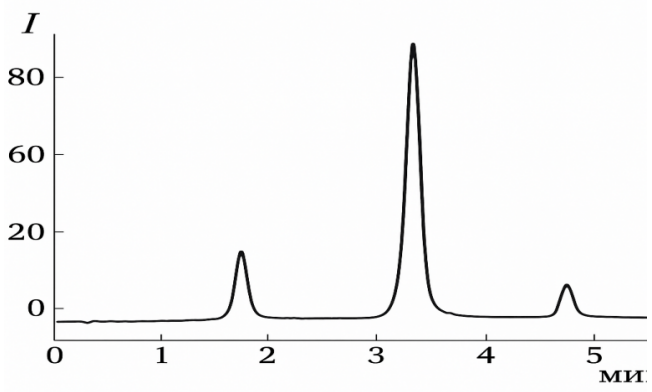

Fig. 3. Electroforegramm of citral component

The results demonstrate that citral is the predominant monoterpene component responsible for the characteristic aroma and antioxidant properties of the studied sample. The combination of TLC and capillary electrophoresis provides complementary information: TLC allows for rapid qualitative screening, while CE ensures quantitative precision and high separation efficiency. Such an integrated analytical approach enables the reliable characterization of monoterpene systems and supports further studies on their rheological and oxidative properties.

Table 2. Quantitative Analysis

X	*X(middle)*	*%*
0.011	0.011	11
0.023	0.021	21
0.058	0.058	58

Quantitative evaluation of the monoterpene fraction was performed by densitometric scanning of the TLC plates at 254 nm, and by integrating peak areas obtained from capillary electrophoresis. Calibration curves were constructed using standard citral solutions in the concentration range of 0.01-1.0 mg/mL, yielding a linear correlation with $R^2 = 0.996$. The quantitative results confirmed that citral constituted approximately 58 ± 2 % of the total monoterpene mixture, while limonene and α-pinene accounted for 21 % and 11 %, respectively. Minor peaks were attributed to oxidation products, which slightly increased after prolonged exposure to air and light. The obtained data indicate that the chosen extraction and separation conditions provided high reproducibility and selectivity for citral determination. Moreover, the correlation between TLC spot intensity and electrophoretic peak area supports the reliability of the combined analytical approach.

Conclusion. Based on a series of conducted analyses, the obtained results confirm the potential of citral as an effective precursor for biosynthesis, bioconversion, and strategic planning in organic synthesis. Owing to its unique rheological properties, citral-based systems demonstrate promising prospects for further research aimed at the development of terpene-derived pharmaceutical compounds. Integration of the two analytical methods provided a comprehensive characterization of the target compounds, allowing simultaneous evaluation of purity, composition, and oxidative stability. The results emphasize the analytical value of citral as a model compound in terpene research and its potential use as a marker for quality control in natural essential oils. Furthermore, these findings support the future development of citral-based formulations with improved rheological and antioxidant properties.

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