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SOLVENT-DEPENDENT CHEMICAL PROFILING AND ANTIMICROBIAL ACTIVITY OF *N*-HEXANE AND DICHLOROMETHANE EXTRACTS DERIVED FROM HYDRODISTILLED ESSENTIAL OIL OF *PHYSALIS ANGULATA* L. FRUITS

Atabek Bayniyazov [a*], Utkir Kurbanov [a], Gulbaxar Tilovova [a], Bakhtiyar Abdurakhmanov [a], Ravshanjon Khalilov [a], Munira Allaeva [b], Jaloliddin Abdurakhmanov [a], Dilmurodjon Turdaliyev [c], Shakhnoz Azimova [a]

[a]*Institute of the Chemistry of Plant Substances, 77, Mirzo Ulugbek str, Tashkent, 100170, Republic of Uzbekistan*
[b]*Tashkent State Medical University, 2, Farabiy str, Tashkent, 100109, Republic of Uzbekistan*
[c]*National Research University "Tashkent Institute of Irrigation and Agricultural Mechanization Engineers Institute", 39, Kari Niyazov str, Tashkent, 100000, Republic of Uzbekistan*
[*]*e-mail: bahti86.86@mail.ru*

Abstract. GC–MS analysis revealed that 59 compounds (96.3%) were identified in the *n*-hexane extract of hydrodistilled essential oil from the fruits of *Physalis angulata* L., with a predominance of fatty acids and their derivatives, including *n*-hexadecanoic acid (21.13%) and conjugated linoleic acid (9.46%). The dichloromethane extract contained 34 compounds (80.15%), mainly oxygenated volatile constituents such as 1-hexanol (24.03%), furfural (6.04%), and 3-hexen-1-ol (5.10%), along with fatty acids. Solvent polarity was shown to play a decisive role in extraction selectivity, with *n*-hexane preferentially extracting lipid components, whereas dichloromethane promoted the extraction of oxygenated volatile compounds. Antimicrobial evaluation demonstrated that the dichloromethane extract exhibited the highest activity against *Bacillus subtilis* (19 ± 0.12 mm), with lower activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, whereas the *n*-hexane extract showed weaker effects. The observed bioactivity can be attributed to the solvent-dependent chemical composition of the extracts, especially the combined effects of fatty acids, alcohols, and aldehydes, potentially mediated by membrane disruption and synergistic interactions. These findings indicate that solvent-dependent fractionation is a key factor in determining the chemical and biological profiles of *P. angulata*.

Keywords: *Physalis angulata*, angular physalis, fruits, essential oil, gas chromatography–mass spectrometry, antimicrobial activity.

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Introduction

Physalis angulata L. (cutleaf groundcherry) is a member of the family *Solanaceae*, which comprises more than 120 species of herbaceous plants with both annual and perennial life forms [1]. *P. angulata* is a bushy annual herbaceous plant that reaches a height of approximately 50 cm. It is characterised by a glabrous or slightly pubescent surface, with simple hairs present [2]. The flowers are bell-shaped, and the fruits are spherical, enclosed in an enlarged calyx that droops as it matures.

The fruits of *P. angulata* are edible and are characterised by pleasant organoleptic properties [3]. The species is widely distributed in tropical and subtropical regions of the world and is known by various common names, including camapu, wild tomato, and winter cherry. The plant is utilised for both culinary and medicinal purposes: the fruits are employed in the preparation of food (including sauces), while the leaves are consumed raw as part of salads [4,5].

In traditional and folk medicine, *P. angulata* is used as a choleretic, diuretic, anti-inflammatory, analgesic, wound-healing, expectorant, and haemostatic agent. The utilization of the fruits in the treatment of diseases of the respiratory, digestive, and endocrine systems, as well as for urolithiasis, gout, and rheumatic conditions, has been documented [6,7]. In certain regions, plant extracts are used as antimalarial and anti-asthmatic agents, in addition to the treatment of dermatological diseases. It has been demonstrated that certain isolated compounds

possess the capacity to exhibit cytotoxic activity against a variety of tumour cell lines [8,9]. *P. angulata* has been demonstrated through experimental studies to possess antiallergic, anti-asthmatic, antileishmanial, antimalarial, and immunomodulatory properties [10-13].

Phytochemical studies indicate a rich metabolic profile of this species. A comprehensive analysis of the plant has revealed the presence of both primary and secondary metabolites, including carbohydrates, lipids, vitamins, mineral elements, and phytosterols. Of particular interest are steroidal lactones of the physalin and withanolide groups (physalins A, I, G; withanolides A, T; 14*α*-hydroxyixocarpanolide; 24,25-epoxywithanolide D; vimonolide; physangulide, *etc*.), as well as flavonoid glycosides, in particular myricetin-3-*O*-neohesperidoside [14-18].

The neutral and polar lipids of the seeds, as well as the fatty acids of the leaves of *P. angulata*, have been characterised. The study established that 12.35% of neutral lipids and 2.18% of polar lipids were isolated from the seeds. The fatty acid composition of the samples was determined, comprising 16–17 components. The leaves of the plant contain 3.6% lipids, and their fatty acid profile is similar to that of the seeds. The leaves contain water-soluble polysaccharides and pectic substances, for which the monosaccharide composition has been determined. A mannan-containing polysaccharide has been identified among the water-soluble polysaccharides of the seeds. The pectic substances of the leaves are represented by highly esterified pectins [19].

It is important to note that volatile components of essential oils (EOs) have been shown to exhibit pronounced biological activity [20] and, in some cases, potential toxicity [21]. Therefore, further investigation of the essential oils of *P. angulata* seeds is a relevant and scientifically justified direction.

The objective of this study was to evaluate the chemical composition of essential oils obtained from the fruits of *Physalis angulata* L., collected in the Parkent district of the Tashkent region, using gas chromatography–mass spectrometry (GC–MS), as well as to determine the influence of solvent polarity on the selectivity of component extraction. Particular emphasis was placed on the comparative analysis of extracts obtained using solvents of different polarity, namely *n*-hexane and dichloromethane. This approach made it possible, for the first time in this region, to reveal specific features in the distribution of fatty acids, oxygenated volatile compounds, and their derivatives in the essential oil composition, thereby expanding current knowledge of the chemical profile and potential biological activity of this species.

Experimental

Materials

All solvents and reagents used were of analytical grade. *n*-Hexane (≥99%) and dichloromethane (≥99.5%) were used for liquid–liquid extraction of the essential oil. Anhydrous sodium sulphate (≥99%) was used for drying organic extracts.

Plant material (*Physalis angulata* L. fruits) was collected in September 2025 (autumn season) in the Parkent district of the Tashkent region, Republic of Uzbekistan (41.180791° N, 69.682981° E). Species identification was performed by PhD in Biological Sciences A. M. Nigmatullaev. A voucher herbarium specimen was deposited and stored at the Institute of the Chemistry of Plant Substances.

Methods

Essential oil isolation

Essential oils (EOs) were obtained from finely ground fruits of *P. angulata*. The plant material was subjected to hydrodistillation at atmospheric pressure for 3 h using a Clevenger-type apparatus according to a standard procedure. The resulting aqueous distillates were successively extracted with *n*-hexane and dichloromethane. The combined organic phases were dried over anhydrous sodium sulphate and filtered. Solvents were removed under reduced pressure.

Prior to analysis, the essential oil samples were stored at 4°C in tightly sealed vials.

Sample description

The obtained essential oils were mobile liquids with a pale yellow colour (seed-derived oils) and a yellow-green colour (pericarp-derived oils). All samples exhibited a characteristic and distinct odour.

Gas chromatography–mass spectrometry (GC-MS) analysis

Qualitative and quantitative analyses of the essential oils were performed by gas chromatography coupled with mass spectrometry (GC–MS). Analyses were carried out using an Agilent 7890 GC system equipped with an Agilent 5975C inert MSD quadrupole mass selective detector.

Separation of components was achieved on an HP-INNOWax fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The oven temperature program was as follows:

initial temperature 60°C (no hold), ramped at 4°C/min to 220°C and held for 10 min, then increased at 10°C/min to 240°C and held for 20 min. The injection volume was 0.2 μL. Hydrogen was used as the carrier gas at a constant flow rate of 1.0 mL/min.

Mass spectra were recorded in electron ionization (EI) mode at 70 eV over a mass range of m/z 10–550 amu.

Compound identification

Compound identification was performed by comparing the obtained mass spectra with those in the Wiley Registry of Mass Spectral Data (9th edition) and the NIST Mass Spectral Library (2017). Retention indices (RI) were calculated relative to a homologous series of *n*-alkanes (C9–C32) under identical chromatographic conditions. Additional confirmation was achieved by comparison of mass spectral fragmentation patterns with literature data [22].

Antimicrobial activity evaluation

The antimicrobial potential of the *n*-hexane and dichloromethane extracts of *Physalis angulata* L. essential oil was assessed against five microbial test strains. The panel included two Gram-positive bacteria, *Bacillus subtilis* RKMUz-5 and *Staphylococcus aureus* ATCC 25923; two Gram-negative bacteria, *Escherichia coli* RKMUz-221 and *Pseudomonas aeruginosa* ATCC 27879; and one fungal strain, *Candida albicans* RKMUz-247. The RKMUz bacterial and fungal cultures were obtained from the Microorganism Culture Collection of the Institute of Microbiology, Republic of Uzbekistan.

The antimicrobial activity of the essential oil extracts and reference drugs was determined using a modified agar disk-diffusion assay. The method was performed according to previously described protocols [28,29], with minor modifications. Briefly, sterile nutrient agar medium was inoculated with the corresponding microbial suspension and poured into Petri dishes under aseptic conditions. After solidification, sterile paper disks (Millipore, Germany) were loaded with 40 μL of each test sample, corresponding to 2 mg of extract per disk, dissolved in methanol.

Ampicillin/Sulbactam (10 μg + 10 μg/disc) was used as the positive control for Gram-positive bacteria, Gentamicin (10 μg/disc) for Gram-negative bacteria, and Fluconazole (25 μg/disc) for fungal strain testing, following antimicrobial susceptibility testing recommendations [28]. All reference antibiotics were obtained from HiMedia Laboratories Pvt. Ltd. Methanol was used as the negative control. Before placement on the inoculated agar surface, the solvent was allowed to evaporate under an air stream.

The prepared disks were carefully placed onto the surface of inoculated agar plates. The plates were maintained at 4°C for 3 h to allow preliminary diffusion of the tested substances into the agar medium. Bacterial cultures were then incubated at 37°C for 12–24 h, whereas fungal cultures were incubated at 26°C for up to 48 h. After incubation, the inhibition zones were measured in millimetres, including the disk diameter. Each assay was performed in three independent replicates, and the mean inhibition zone was calculated.

Statistical analysis

All experiments were carried out in triplicate. The obtained data were processed using OriginPro software and expressed as mean ± standard deviation (SD). Statistical significance was considered at $P \leq 0.05$.

Results and discussion

Chemical Composition of Physalis angulata Essential Oil

The essential oil (EO) of *P. angulata* fruits was subjected to gas chromatography–mass spectrometry (GC–MS) analysis, which resulted in the identification of 59 individual compounds (see Table 1 for details). Subsequent extraction of the residual phase with dichloromethane enabled the identification of a further 34 compounds (Table 2).

A comparative analysis of the *n*-hexane and dichloromethane extracts was conducted, revealing significant discrepancies in both quantitative and qualitative composition (Tables 1 and 2). In the *n*-hexane extract, 59 compounds were identified, with a total content of 96.3% (see Table 1), whereas in the dichloromethane extract, 34 components were identified (80.15%) (see Table 2). It is important to note that in this study, a sequential extraction of the distillate was employed: initially with *n*-hexane, followed by the extraction of the remaining phase with dichloromethane. Consequently, the dichloromethane extract contains components not extracted by hexane, thereby indicating the incomplete selectivity of hexane and the presence of compounds with intermediate and higher polarity remaining in the system after the initial extraction stage.

Table 1

Identified components of the essential oil of *P. angulata* fruits obtained by *n*-hexane extraction.

Nr.	*Components*	*RI*	*Content, %*
1.	1-Hexanol	1253.3	0.18
2.	Nonanal	1319.7	0.78
3.	2,6,10-Trimethyltridecane	1455.4	0.14
4.	Decanoic acid, methyl ester	1521.7	0.10
5.	2-Undecanone	1529.4	0.42
6.	Alloaromadendrene	1568.9	0.17
7.	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	1662.5	0.52
8.	Gamma-muurolene	1666.3	1.29
9.	2,4-Decadienal	1725.5	0.28
10.	Octadecane	1797.4	0.16
11.	Alpha-calacorene	1800.6	0.23
12.	1H-Indene, 2,3-dihydro-1,1,5,6-tetramethyl-	1892.6	0.10
13.	Nonadecane	1899.7	0.29
14.	Longifolenaldehyde	1922.6	0.16
15.	Naphthalene	1939.4	0.31
16.	2-Dodecen-1-yl(-)succinic anhydride	1954.6	0.15
17.	Azulene	1961.7	3.91
18.	2-Methoxy-4-vinylphenol	2035.6	0.57
19.	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	2045.5	0.35
20.	(8*R*,8a*S*)-8,8a-Dimethyl-2-(propan-2-ylidene)-1,2,3,7,8,8a-hexahydronaphthalene	2055.5	0.21
21.	Nonadecane	2098.8	0.76
22.	*n*-Decanoic acid	2122.8	6.73
23.	Hexadecanoic acid, methyl ester	2136.7	2.05
24.	Isopropyl 9-hexadecenoate	2147.1	0.18
25.	Hexadecanoic acid, ethyl ester	2180.9	3.56
26.	Ethyl 9-hexadecenoate	2190.0	0.36
27.	Heptadecane	2197.9	0.25
28.	Tricosane	2300.6	1.56
29.	Dodecanoic acid	2336.6	3.42
30.	Methyl stearate	2345.4	0.20
31.	11-Octadecenoic acid, methyl ester	2350.1	0.65
32.	1-Docosene	2370.5	0.20
33.	9,12-Octadecadienoic acid (*Z*,*Z*)-, methyl ester	2384.1	2.69
34.	(*E*)-9-Octadecenoic acid, ethyl ester	2392.6	1.20
35.	Tetracosane	2399.7	0.55
36.	Linoleic acid, ethyl ester	2425.0	10.28
37.	9,12,15-Octadecatrienoic acid, methyl ester	2436.7	0.51
38.	Bicyclo[10.1.0]tridec-1-ene	2443.3	0.29
39.	3-Eicosene	2479.9	0.23
40.	Pentacos-1-ene	2500.3	0.99
41.	1-Naphthalenepropanol	2517.5	6.19
42.	Tetradecanoic acid	2548.6	1.63
43.	(*E*)-Hexacosane	2598.4	0.33
44.	Pentadecanoic acid	2655.5	0.43
45.	3-Eicosene	2688.4	0.15
46.	Heptacosane	2701.3	0.57
47.	*n*-Hexadecanoic acid	2757.1	21.13
48.	*cis*-7-Hexadecenoic acid	2779.3	1.56
49.	Cetene	2799.1	0.39
50.	Cyclotridecane	2867.4	0.22
51.	1-Docosene	2899.6	0.74
52.	1-Heptadecene	2909.9	0.21
53.	Octadecanoic acid	2969.5	1.04
54.	9-Octadecenoic acid	2987.8	2.97
55.	Oleyl alcohol	2995.8	0.77
56.	10(*E*),12(*Z*)-Conjugated linoleic acid	3069.4	9.46
57.	9,12,15-Octadecatrienoic acid	3130.5	1.53
	Total		**96.3**

For the *n*-hexane extract, the dominance of fatty acids and their derivatives is characteristic (Table 1). The main component is n-hexadecanoic (palmitic) acid (21.13%), indicating the predominance of saturated fatty acids in the oil composition. Palmitic acid is known for its membranotropic activity and moderate antimicrobial properties [23]. Saturated fatty acids, in general, exhibit pronounced antimicrobial activity due to their ability to disrupt the permeability of cytoplasmic membranes of microorganisms [24,25].

Along with saturated fatty acids, significant amounts of unsaturated fatty acids and their derivatives were identified in the EO of *P. angulata* fruits. In particular, linoleic acid and its esters were identified, including the ethyl ester (10.28%), as well as 10(*E*),12(*Z*)-conjugated linoleic acid (9.46%) (Table 1). It is known that conjugated isomers of linoleic acid are capable of modulating lipid metabolism and influencing inflammatory signalling pathways [26]; therefore, the presence of these compounds may indicate the potential antioxidant and anti-inflammatory activity of the studied oil.

In addition, the presence of oleic acid and its derivatives was confirmed (see Tables 1 and 2), which further validates the essential oil's (EO) high biological potential and enhances our understanding of its pharmacologically significant properties.

The minor components of the *n*-hexane extract (Table 1) are pentacos-1-ene (0.99%), oleyl alcohol (0.76%), nonadecane (0.76%), methyl 11-octadecenoate (0.65%) and heptacosane (0.57%). The presence of 11-octadecenoic acid and its derivatives may enhance the EO's anti-inflammatory and antioxidant potential.

Table 2

Component composition of the essential oil of *Physalis angulata* fruits identified by dichloromethane extraction.

Nr.	*Components*	*RI*	*Content, %*
1.	3,6-Dimethyl-2-pentanol	1206	1.44
2.	1-Hexanol	1248	24.03
3.	3-Hexen-1-ol	1268	5.10
4.	2-Hexen-1-ol	1294	1.45
5.	Furfural	1339	6.04
6.	3-Furaldehyde	1352	1.43
7.	Benzaldehyde	1430	0.27
8.	Cyclohexene	1600	0.57
9.	Naphthalene	1671	1.14
10.	(+)-cis-Verbenol	1704	0.46
11.	Benzene	1720	0.79
12.	Azulene	1967	2.26
13.	Sulfurous acid	2099	0.32
14.	*n*-Decanoic acid	2127	4.89
15.	Hexadecanoic acid, methyl ester	2139	1.08
16.	Hexadecanoic acid, ethyl ester	2183	1.52
17.	Heptacosane	2299	0.71
18.	Dodecanoic acid	2340	1.48
19.	11-Octadecenoic acid, methyl ester	2352	0.26
20.	8,11-Octadecadienoic acid, methyl ester	2386	0.92
21.	Ethyl oleate	2393	0.46
22.	9,12-Octadecadienoic acid, ethyl ester	2428	4.47
23.	(Z,Z,Z)-6,12,15-Octadecatrienoic acid, methyl ester	2441	0.19
24.	Cetene	2499	0.38
25.	Bicyclo[3.1.1]heptane	2515	0.28
26.	Phenanthrene	2522	2.92
27.	1-Tridecene	2551	0.57
28.	*n*-Hexadecanoic acid	2760	9.02
29.	7-Tetradecene	2786	0.58
30.	1-Heptadecene	2973	0.40
31.	Oleic acid	2991	1.50
32.	9,12-Octadecadienoic acid	3078	3.22
	Total		**80.15**

Table 3

Antibacterial and antifungal activity of *Physalis angulata* L. essential oil extracts evaluated by inhibition zone diameter in the agar disk-diffusion assay.

Compound	*Gram-positive bacteria*		*Gram-negative bacteria*		*Fungi*
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
n-Hexane extract	8 ±0.11	na	na	6 ±0.10	6 ±0.11
Dichloromethane extract	19 ±0.12	7	na	6 ±0.12	8 ±0.10
Ampicillin/Sulbactam	28 ±0.10	26 ±0.10	nt	nt	nt
Gentamicin	nt	nt	19 ±0.10	22 ±0.10	nt
Fluconazole	nt	nt	nt	nt	25 ±0.11

Data are presented as mean ± SD, $P \leq 0.05$. *na* = not active; nt* = not tested.*

The combination of the identified compounds, which include saturated and unsaturated fatty acids, their esters and hydrocarbon components, suggests the possibility of a synergistic effect determining the overall biological activity of the plant material under study [27].

Unlike the *n*-hexane extract, the dichloromethane sample (Table 2) is dominated by low-molecular-weight, oxygen-containing compounds, primarily 1-hexanol (24.03%), as well as 3-hexen-1-ol (5.10%) and furfural (6.04%). Nevertheless, a significant proportion of fatty acids is retained, including *n*-hexadecanoic acid (9.02%) and *n*-decanoic acid (4.89%).

Therefore, the polarity of the solvent plays a decisive role in the selectivity of component extraction: *n*-hexane predominantly concentrates fatty acid fractions, whereas dichloromethane facilitates the extraction of more volatile alcohols and aldehydes.

Antimicrobial activity of the n-hexane and dichloromethane extracts of Physalis angulata L. essential oil

The antimicrobial activity of the *n*-hexane and dichloromethane extracts of *Physalis angulata* L. essential oil is summarized in Table 3.

Among the tested samples, the dichloromethane extract exhibited the most pronounced inhibitory activity, particularly against *Bacillus subtilis*, with an inhibition zone of 19 ± 0.12 mm. A weaker effect was observed against *Staphylococcus aureus* and *Candida albicans*, with inhibition zones of 7 mm and 8 ± 0.10 mm, respectively. The extract also produced a small inhibition zone against *Pseudomonas aeruginosa* (6 ±0.12 mm), whereas no activity was detected against *E. coli*. The relatively stronger antimicrobial effect of the dichloromethane extract may be associated with its chemical profile, especially the presence of 1-hexanol [30], furfural, 3-hexen-1-ol, decanoic acid, and palmitic acid [31]. These compounds have previously been linked to antimicrobial activity, including effects related to membrane disruption and altered microbial cell permeability [32]. However, the exact contribution of each individual compound requires further investigation.

The *n*-hexane extract demonstrated moderate activity against selected microorganisms. It inhibited *Bacillus subtilis* with a zone of 8 ± 0.11 mm and showed weak inhibition against *Pseudomonas aeruginosa* and *Candida albicans*, both with zones of 6 mm. No inhibitory effect was observed against *Staphylococcus aureus* or *E. coli*. This moderate activity may be related to the high content of fatty acids, including palmitic, linoleic, conjugated linoleic, and oleic acids [33].

Although the precise antimicrobial mechanism of six-carbon alcohols and fatty acids has not been fully clarified, previous studies suggest that these compounds may act through membrane-associated mechanisms, leading to changes in membrane integrity, increased permeability, and leakage of intracellular components [34]. In addition, the antimicrobial activity of essential oils is often not attributable to a single constituent alone, but may result from additive or synergistic interactions among multiple volatile and non-volatile components [35]. Therefore, the observed activity of *P. angulata* essential oil extracts may reflect the combined effects of alcohols, aldehydes, fatty acids, and other minor constituents present in the extracts.

Conclusions

GC–MS analysis of the essential oils from *Physalis angulata* fruits allowed the identification of 59 compounds, accounting for the major fraction of the extracts (96.3% in the *n*-hexane extract and 80.15% in the dichloromethane extract). It was shown that the chemical composition strongly depends on solvent polarity, confirming the

efficiency of sequential extraction for fractionation of components.

The *n*-hexane extract was characterized by the predominance of fatty acids, whereas the dichloromethane extract was enriched with low-molecular-weight oxygenated compounds (alcohols and aldehydes), while still retaining fatty acid constituents.

The observed features of the chemical composition expand current knowledge of the phytochemical profile and potential biological activity of this species and highlight its promise as a source of bioactive compounds for applications in the pharmaceutical and food industries.

The results showed that the dichloromethane extract of *Physalis angulata* L. essential oil exhibited stronger antimicrobial activity than the *n*-hexane extract, particularly against *Bacillus subtilis* (19 ± 0.12 mm). Moderate or weak inhibitory effects were also observed against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, whereas *Escherichia coli* was resistant to both extracts. The observed antimicrobial activity may be associated with the combined effects of alcohols, aldehydes, fatty acids, and other bioactive constituents, possibly through membrane-related mechanisms.

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