

## Physiologically active compounds in the fruits of *Smilax excelsa* L.

Vafa Atayeva<sup>1\*</sup>, Farhad Azizov<sup>1</sup>, Zarbali Khalilov<sup>1</sup>, Nurmammad Mustafayev<sup>2</sup>, Hilal Imanli<sup>3</sup>

<sup>1</sup>Azerbaijan National Academy of Sciences, Sheki Regional Scientific Centre, Sheki, Azerbaijan

<sup>2</sup>Ministry of Science and Education of the Republic of Azerbaijan, Institute of Molecular Biology and Biotechnologies, Baku, Azerbaijan

<sup>3</sup>Azerbaijan National Academy of Sciences, Institute of Botany, Baku, Azerbaijan

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**Abstract:** Currently, in all areas, especially in the fields of food and medicine, eco-friendly consumption materials are highly preferred. Therefore, the study aims to determine the physiologically active compounds and radical scavenging activity (RSA) of the bioextracts prepared with 70% ethanol (BE-I) and distilled water (BE-II) from the ripe fruits of *Smilax excelsa* L. (Smilacaceae) plant which grows in the northwestern region of Azerbaijan by physicochemical methods ((GC/MS), ultraviolet (UV) spectrophotometers). As a result of the research, 35 compounds were observed in BE-I and 30 compounds in BE-II, the amount of main physiologically active compounds was 37.14% with 12 compounds in BE-I, and 19.86% with 6 compounds in BE-II. For instance, antioxidant properties of 5-hydroxymethylfurfural (C<sub>6</sub>H<sub>6</sub>O<sub>3</sub>), 6-Octadecenoic acid (C<sub>18</sub>H<sub>34</sub>O<sub>2</sub>), hepta-2,4-dienoic acid, methyl ester (C<sub>8</sub>H<sub>12</sub>O<sub>2</sub>), antibacterial traits of ascaridole epoxide (C<sub>10</sub>H<sub>16</sub>O<sub>3</sub>), α-D-Galactopyranose,6-O-(trimethylsilyl)-cyclic 1,2:3,4-bis methylboronate (C<sub>11</sub>H<sub>22</sub>B<sub>2</sub>O<sub>6</sub>Si), hexadecanoic acid, methylester (C<sub>17</sub>H<sub>34</sub>O<sub>2</sub>), flavoring compounds of 2H-Pyran, 3,6 Dihidro-4-methyl-2-2-Methyl-propenyl (C<sub>10</sub>H<sub>16</sub>O), E-10-Dodecen-1-ol propionate (C<sub>15</sub>H<sub>28</sub>O<sub>2</sub>), anticancer properties of 2-Trifluoroacetoxydodecane (C<sub>14</sub>H<sub>25</sub>F<sub>3</sub>O<sub>2</sub>), anti-inflammatory properties of silicic acid, diethyl bis(trimethylsilyl) ester (C<sub>10</sub>H<sub>28</sub>O<sub>4</sub>Si<sub>3</sub>), antifungal traits of D-Mannitol,1-decylsulfonyl (C<sub>16</sub>H<sub>34</sub>O<sub>7</sub>S) were determined. The DPPH method was used to determine the free radical scavenging activity (RSA), which came out to be RSA<sub>(BE-I)</sub>=62.5±0.8% and RSA<sub>(BE-II)</sub>=54.7±1.3%.

## 1. INTRODUCTION

*Smilax excelsa* L. (Smilacaceae), one of the economically and medicinally important plants, is found in the northwest of Azerbaijan. Physiologically active chemicals and pharmacological qualities of *Smilax excelsa* L. fruits grown in Azerbaijan have not been studied. One species of *Smilax excelsa*, a liana-type plant, primarily grows in the Caucasus and Azerbaijan. The leaves, fruits, and roots of this plant have been used medicinally for centuries by various cultures. Additionally, shamans traditionally used it to treat dermatitis, psoriasis, and other skin conditions (Əsgərov, 2005). People in Central and South America utilized the root of this plant as a diuretic and tonic booster (Əzizov *et al.*, 2020; Elina, 1993).

\*CONTACT: Vafa ATAYEVA ✉ [vefaatayeva81@gmail.com](mailto:vefaatayeva81@gmail.com) 📍 Azerbaijan National Academy of Sciences, Sheki Regional Scientific Center, Sheki Azerbaijan

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Currently, this plant's leaves, roots, and fruits are frequently used in scientific medicine to treat and avert diseases of the gastrointestinal tract (Cox *et al.*, 2005), genitourinary (Pansumrit *et al.*, 2022), nervous system disorders, and depression (Sayyaed *et al.*, 2023) in the body. The presence of biologically active steroid saponins in the formulation of the remedies made from different plant parts accounts for their therapeutic action. Extracts of *Smilax excelsa* L. can be used to treat conditions affecting the kidneys, bladder, urinary system, pancreas, liver, and biliary tract (Bruno *et al.*, 1985; Muravyova, 1981).

Many species of *Smilax excelsa* found in different parts of the world have been studied for their chemical composition. It was found that these species are abundant in physiologically active chemicals with notable antioxidant properties (Kretovich, 1989). Antioxidant capacity and components of *Smilax* species gathered from various locations by Turkish researchers were determined, in addition to certain phenological and morphological aspects. They concluded that *Smilax* species' leaves and berries can be regarded as a significant source of naturally occurring antioxidants (Yildiz *et al.*, 2018). Later the anthocyanin content of its fruit was studied, and pelargonidin 3-O-rutinoride ( $20.4 \text{ mg}^{-1}$ ) and cyanide 3-O-rutinoride ( $1.67 \text{ mg}^{-1}$ ) were discovered to be the major anthocyanins (Longo & Vasapollo, 2006). *Smilax excelsa* leaves, which have spread widely in the Black Sea region of Turkey, were found to have antioxidant properties. The antioxidant potential of the extracts was also assessed, and measures were taken for lipid peroxidation inhibition, metal ion chelation, DPPH, reduction of superoxide, hydroxyl radicals, and hydrogen peroxide, as well as radical scavenging competencies (Ozsoy *et al.*, 2008). By comparing the antioxidant behaviors of water, infusion, ethanol, and ethyl acetate extracts to their total amount of polyphenol components, the extraction method's efficiency was calculated (Chen *et al.*, 2000). Traditional Chinese medicine frequently uses the root and leaf of the *Smilax China* plant, an indigenous species of the *Smilax* genus, to treat inflammatory pain, rheumatic, diuretics, joint pain, and gout (Khan *et al.*, 2009). In subsequent studies, the content of phenolic compounds in the root, stem, and leaf of *Smilax China* was 19.31%, 8.59%, and 44.27% respectively, in which 3-hydroxy benzoic acid predominated as the major phenolic compound and DPPH activity of the extract was determined to be 8.66 mg/ml, and the total phenolic content was 2,383.55  $\mu\text{g}$  gallic acid equivalent/mL (Shim, 2012). Previous research determined the presence of 25 mineral elements in the vegetative and generative organs of the *Smilax excelsa* L. plant, the amount of microelements Zn, Mn, Cr, Si was 0.679% in the leaf, 0.935% in the stem, 2.10% in the flower and 0.578% in the fruit. The fruits of the *Smilax* genus have been found to contain a high concentration of physiologically active compounds based on their biochemical composition, including carotene (0.69 mg%), carotenoids (7.35 mg%), ascorbic acid (103 mg%),  $\alpha$ -tocopherol (5.62 mg%), and flavonoids (0.90%) (Xəlilov & Xəlilov, 2021). Other researchers prefer mainly the root and stem parts of the plant. This causes considerable damage to the reduction of plant biodiversity and the ecosystem chain. The most commonly used part of this plant in folk medicine and household are the dry fruits of this plant. Therefore, we prefer to conduct research with fruit instead of working with other vegetative organs.

In light of the above, this significant plant native to Azerbaijan should be thoroughly studied to identify its physiologically active compounds and conduct research that could facilitate its application in scientific medicine.

## 2. MATERIAL and METHODS

### 2.1. Extract Preparation

For the experiment, ripe fruits of *Smilax excelsa* which belongs to the northern-west region of Azerbaijan were collected. The fruits were first washed with water, separated from seeds, dried, and then crushed. Then the crushed materials were subjected to extraction using distilled water (BE-II) and 70% ethanol (BE-I). A 500-milliliter flask was filled with a 50 gram of ground sample. Subsequently, the sample was mixed with 300 milliliters of distilled water. The mix was incubated for 30 minutes at a temperature of between 75 and 80°C in a water bath, after which the solution was filtered. Then, the remaining pulverized sample was mixed with 100

mL of distilled water and extracted for 15 minutes. After extraction, it was filtered and combined with the original extract. The residue in the flask was refilled with 100 mL of distilled water and extracted for an additional 15 minutes. It was extracted, filtered, and then mixed with the first extract. The extraction process in alcohol also was carried out in the same way. A device known as an SPT-200 Vacuum-Drier was used to powder both extracts (Azizov *et al.*, 2021).

Taking into account the preparation of plant extracts at home, it is appropriate to prepare bioextracts with water and ethanol, because they are available to the population and their preparation is simple. Although other organic solvents are more effective, the use of this method for the population is inappropriate and does not guarantee safety.

## 2.2. Identification of Organic Compounds

Biologically active compounds in the extracts were identified by using an Agilent Technologies 6890 N Network CG System, a gas chromatograph equipped with a 5975 inert Mass Selective Detector spectrometer (USA). It also has an injection-split detector that was split/splitless, split - 100, low mass – 40, high mass – 400, and threshold 150. A 30-meter quartz capillary column named "HP-5MS 5% Methyl Siloxane" with an internal diameter of 0.25 mm and a stationary phase thickness of 0.25 $\mu$ m was used for the experiments. The analyses were carried out in temperature programming mode at a rate of 15°C per minute between 50°C and 280°C. The temperature regime in the column was as follows: the beginning temperature of 50°C that was constant for two minutes; a two-minute temperature rise from 15°C to 200°C; a ten-minute temperature rise from 15°C to 280°C; and a vacuum HiVac of 3.38e-005. Dilution was carrying by using a 1:1:2 methanol, chloroform to water combination. The velocity of He gas was 1 milliliter per minute. Materials were identified using the standard mass spectroscopic NIST library. In total 33 minutes were spent on the analysis (Azizov *et al.*, 2022).

## 2.3. Radical Scavenging Activity (RSA) assay

The free radical scavenging activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Sigma-Aldrich, USA). A solution of 70  $\mu$ M 2,2-diphenyl-1-picrylhydrazyl (DPPH) was produced in methanol, and the absorption was then measured in a 3 ml cuvette using a UV-2700(Shimadzu, Japan), and the value of absorption was roughly 0.8211 at 516 nm. After extracting 0.5 ml of DPPH solution from that cuvette and adding 0.5 ml of 200 mgL<sup>-1</sup> bioextract, the kinetics was observed after 20 minutes. The following formula was used to determine the DPPH radical scavenging activity: RSA% is equal to  $(A_0 - A_s)/A_0$  (Atayeva & Aslanov, 2022).

## 2.4. Statistical Analysis

The mean values of triplicates of the same sample comprise the results, and analysis of variance (ANOVA) was used for statistical analysis and differences between them consisted of  $p < 0.05$

## 3. FINDINGS

Following the experiment, GC/MS was used to record 35 chemicals in BE-I and 30 compounds in BE-II. During the experiment, GC/MS recorded 35 chemical compounds in BE-I and 30 chemical compounds in BE-II. Each compound was identified individually and their medicinal characteristics were defined and chosen based on the literature that was accessible. In BE-1 and BE-2, the physiologically active chemicals that were selected comprised 37.14% and 19.86%, respectively. Physiologically active substances in the BE-I extract are listed in Table 1, and physiologically active compounds in the BE-II extract are given in Table 2, as no information was discovered about additional substances in the extracts. The mass spectrum results of the identified physiologically active compounds are shown in Figure 1.

**Table 1.** Physiologically active chemicals in contents of BE-I.

Peaks	RT	Compound name	Formula of compound	m/z	Peak area %	Therapeutic activity
1	6.940	2H-Pyran,3,6 Dihydro-4-methyl-2-(2-Methyl-propenyl)	C <sub>10</sub> H <sub>16</sub> O	98.07	5.42	Aromatic, flavoring (Karabagias <i>et al.</i> , 2021)
2	7.240	2-Trifluoroacetoxydodecane	C <sub>14</sub> H <sub>25</sub> F <sub>3</sub> O <sub>2</sub>	282.18	0.47	Anticancer (Fadeyi <i>et al.</i> , 2015)
3	10.369	5-Hydroxymethylfurfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.03	20.19	Anticancer(Kelleci & Gölebatmaz, 2023; Antioxidant (Zhao <i>et al.</i> , 2013)
4	10.575	6-Acetyl-β-d-mannose	C <sub>8</sub> H <sub>14</sub> O <sub>7</sub>	222.07	1.02	Antimicrobial (Ezekwe & Chikezie, 2017)
5	11.004	4-Hydroxylamino-6-methylpyrimidin-2(1H)-one	C <sub>5</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub>	141.05	41.19	Anticancer, anti-oxidant, anti-tumorigenic, anti-mutagenic, cytotoxic activity (Mohan <i>et al.</i> , 2018)
6	11.257	Ascaridole epoxide	C <sub>10</sub> H <sub>16</sub> O <sub>3</sub>	184.11	2.56	Antioxidant, antimicrobial, anticancer (El-Amier <i>et al.</i> , 2023)
7	13.716	α-D-Galactopyranose,6-O-(trimethylsilyl)-cyclic 1,2:3,4-bis(methylboronate)	C <sub>11</sub> H <sub>22</sub> B <sub>2</sub> O <sub>6</sub> S	300.14	0.17	Antimicrobial (Shelly <i>et al.</i> , 2015)
8	18.021	Carbamic acid, 3-pentylidene-, methyl ester	C <sub>7</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>	158.11	1.26	Hypertension, vasodilator (Liu <i>et al.</i> , 2009)
9	19.892	E-10-Dodecen-1-ol propionate	C <sub>15</sub> H <sub>28</sub> O <sub>2</sub>	240.21	0.12	Aromatic, flavoring Mishra <i>et al.</i> , 2021
10	20.686	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.26	1.05	Antimicrobial (Ezekwe & Chikezie, 2017), Antifungal(Francis <i>et al.</i> , 2021), Antibacterial (Shaaban <i>et al.</i> , 2021), Anti-Inflammatory,(Othman <i>et al.</i> , 2015) Antioxidant (Muflihunna <i>et al.</i> , 2019)
11	26.791	D-Mannitol,1-decylsulfonyl	C <sub>16</sub> H <sub>34</sub> O <sub>7</sub> S	370.20	1.68	Antimicrobial (Khromykh <i>et al.</i> , 2022), Antifungal (Thankaraj <i>et al.</i> , 2020), Antibacterial (Khromykh <i>et al.</i> , 2022), Anti-inflammatory (Khromykh <i>et al.</i> , 2022), Antioxidant (Khromykh <i>et al.</i> , 2022)
12	29.497	Silicic acid, diethyl bis(trimethylsilyl) ester	C <sub>10</sub> H <sub>28</sub> O <sub>4</sub> Si <sub>3</sub>	296.13	0.3	Antibacterial, (Momin & Thomas, 2020)

**Table 2.** Physiologically active chemicals in contents of BE-II.

Peaks	RT	Compound name	Formula of compound	m/z	Peak area%	Therapeutic behavior
1	8.981	Levogluconone	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.03	2.35	Anticancer, antitumor (Delbart <i>et al.</i> , 2022)
2	9.457	Hepta-2,4-dienoic acid, methyl ester	C <sub>8</sub> H <sub>12</sub> O <sub>2</sub>	140.08	25.36	Antioxidant, antiproliferative (Fagbemi <i>et al.</i> , 2022)
3	10.445	5-hydroxymethylfurfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.03	32.62	Anticancer (Kelleci & Gölebatmaz, 2023), Antioxidant (Zhao <i>et al.</i> , 2013)
4	11.939	7-Methyl-Z-tetradecen-1-ol acetate	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	268.24	1.26	Antimicrobial, antifungal, antioxidant (Fagbemi <i>et al.</i> , 2022)
5	20.803	Hexadecanoic acid, methylester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.26	3.41	Antimicrobial, (Ezekwe & Chikezie, 2017), Antifungal (Francis <i>et al.</i> , 2021), Antibacterial (Shaaban <i>et al.</i> , 2021), Anti-inflammatory, (Othman <i>et al.</i> , 2015), Antioxidant (Muflihunna <i>et al.</i> , 2019)
6	27.168	6-Octadecenoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.25	14.62	Antioxidant (Alaribe <i>et al.</i> , 2020)

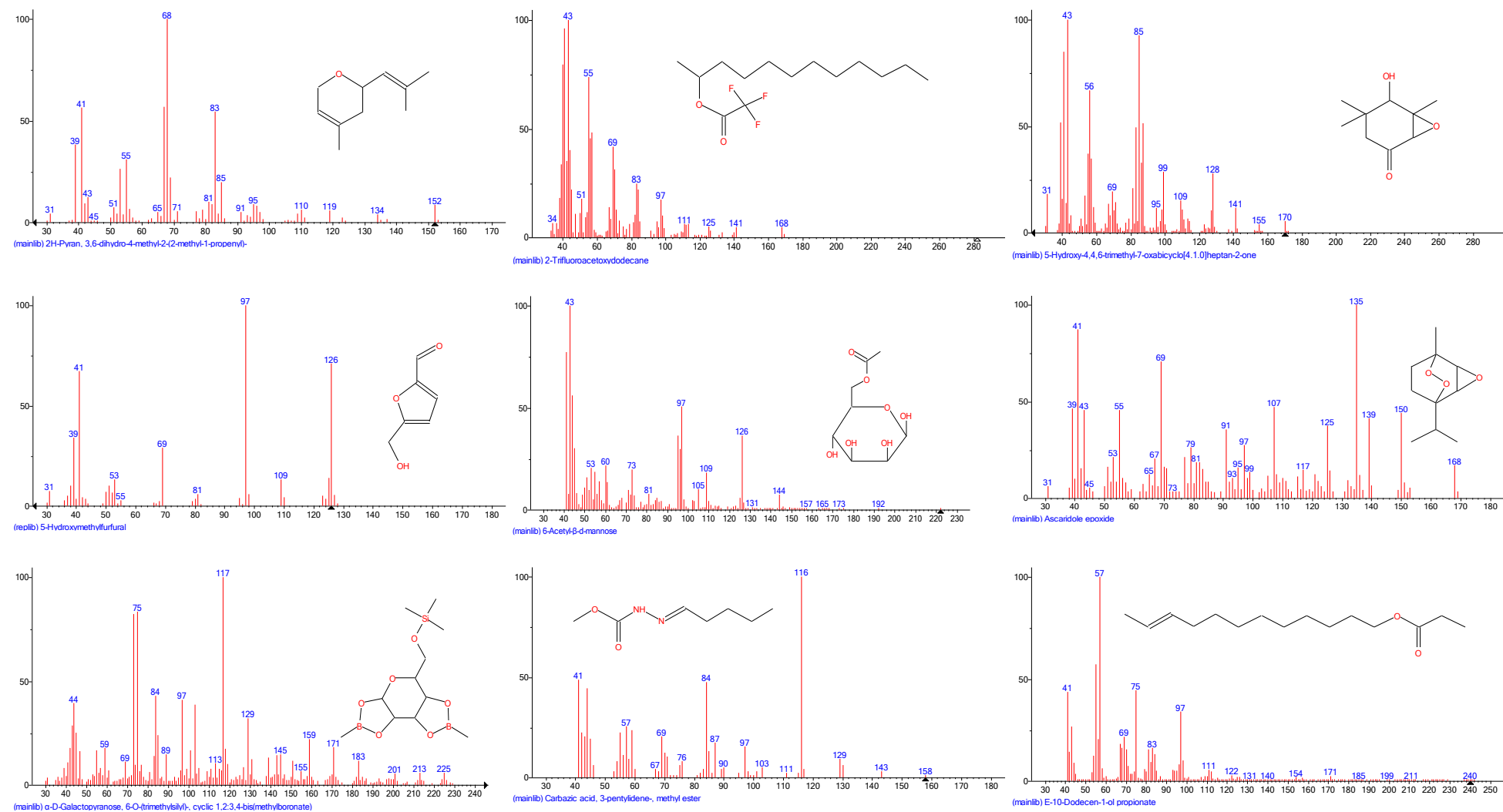


Figure 1. Mass spectra of physiologically active compounds shown in Table 1 and Table 2.

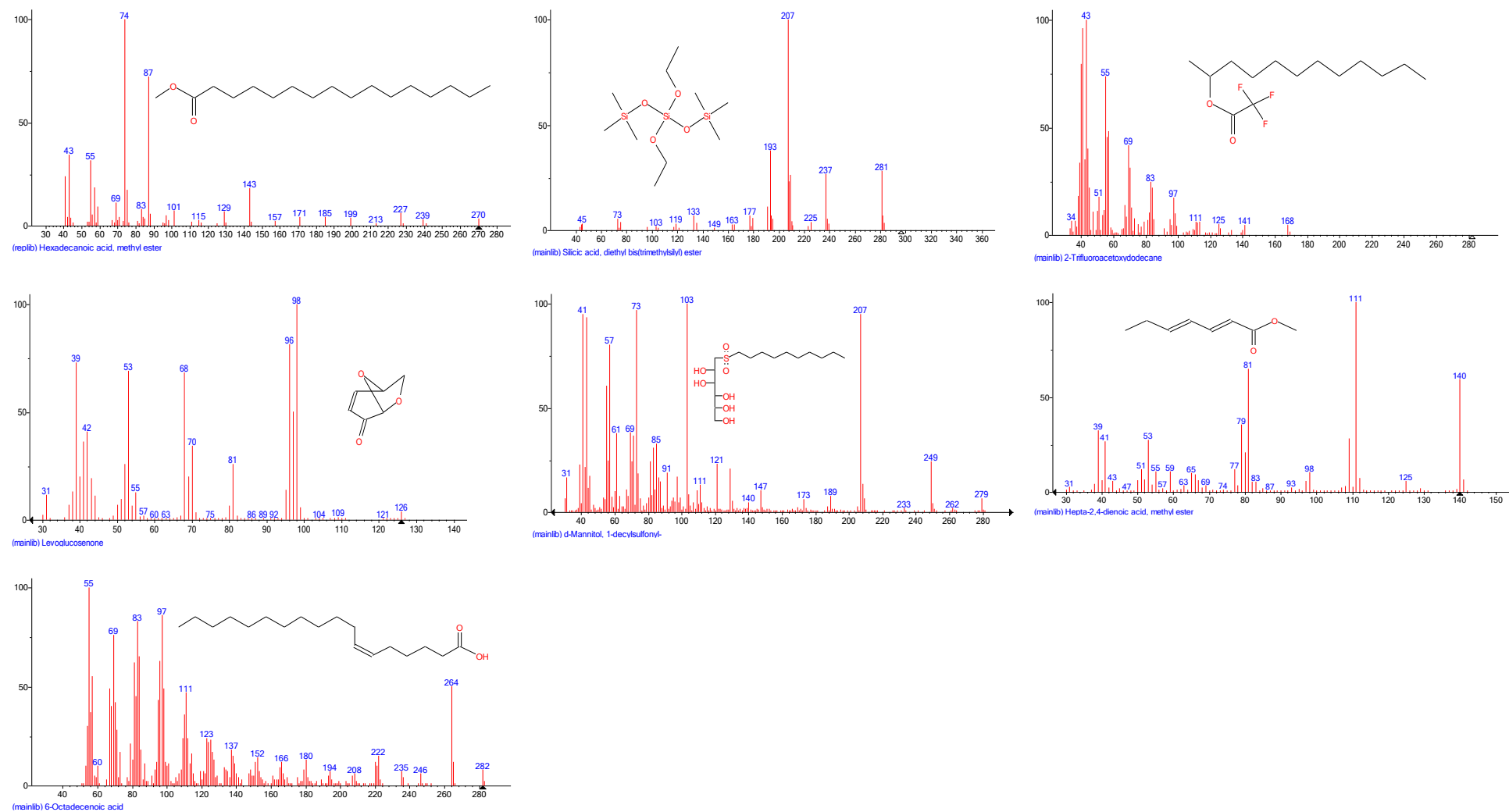


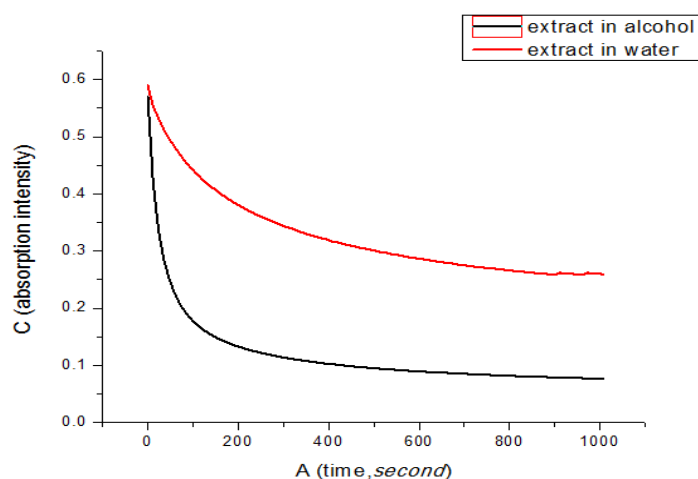
Figure 1. Continues.



The outcomes of the investigation revealed that the bioextract made with 70% ethanol (BE-I) consisted of 12 physiologically active compounds, however, the bioextract made with distilled water (BE-II) contained 6 physiologically active compounds. Physiologically active compounds, which in their separate states have substantial medical value in the body, were present in both bioextracts. These molecules exhibit distinct physiological implications. The most often utilized chemical in the production of nutmeg syrup is 2H-Pyran,3,6 Dihydro-4-methyl-2-(2-Methyl-propenyl), also called nerol oxide. This chemical, which is mostly present in flowers and gives them a sweet, pleasant scent, is produced when the monoterpene nerols oxidize. It was previously employed in the manufacture of wine (Karabagias *et al.*, 2021). Furthermore, it was discovered that flavoring ingredient E-10-Dodecen-1-ol propionate is a considerable component (Mishra *et al.*, 2021). With a high peak area in both extracts, 5-hydroxymethylfurfural exhibits anticancer and antioxidant qualities (Kelleci & Gölebatmaz, 2023; Zhao *et al.*, 2013). According to earlier research, *Smilax excelsa* fruits contain considerable levels of 6-Acetyl- $\beta$ -d-mannose, which has been shown to have antibacterial properties (Ezekwe & Chikezie, 2017). Indian scientists conducted research that led to the discovery of 4-Hydroxylamino-6-methylpyrimidin-2(1H)-one, a chemical with anticancer, antioxidant, anti-tumorigenic, anti-mutagenic, and cytotoxic characteristics (Mohan *et al.*, 2018). Research by Nouf S. Zaghloul and others has demonstrated the antioxidant, antibacterial, and anticancer effects of Ascaridole epoxide, the predominant component of the *Smilax excelsa* fruit extract (El-Amier *et al.*, 2023). In comparison to amoxicillin, the antimicrobial activity of  $\alpha$ -D-Galactopyranose,6-O-(trimethylsilyl)-cyclic 1,2:3,4-bis(methylboronate) encountered in BE-1 against gram positive bacteria demonstrates that it possesses beneficial antibacterial properties and could potentially be used for medicinal purposes in the future (Shelly *et al.*, 2015.). The following substances have antioxidant, antimicrobial, antifungal, anti-inflammatory and antibacterial properties: hexadecanoic acid, methyl ester, D-mannitol,1-decylsulfonyl (Francis *et al.*, 2021; Khromykh *et al.*, 2022; Muflihunna *et al.*, 2019; Othman *et al.*, 2015; Shaaban *et al.*, 2021; Thankaraj *et al.*, 2020). Vasodilators and hypertension are two illnesses for which carbazic acid,3-pentylidene, methyl ester is utilized (Ezekwe & Chikezie, 2017; Parveen *et al.*, 2013). Levoglucosenone, which was identified in BE-2, and 2-Trifluoroacetoxy dodecane, which was found in BE-1, both have anticancer and antitumor characteristics (Delbart *et al.*, 2022; Fadeyi *et al.*, 2015). While examining the *Dillenia scabrella* species leaves and bark, Monim *et al.* found Silicic acid diethyl bis(trimethylsilyl) ester, and highlighted the plant's antibacterial properties (Momin & Thomas, 2020.). Because of its antifungal properties, 7-Methyl-Z-tetradecen-1-ol acetate can lower fungus biomass by 36–54%. High levels of antibacterial, antioxidant, and antiproliferative activity were demonstrated by Hepta-2,4-dienoic acid, methyl ester, 6-Octadecenoic acid, and 7-Methyl-Z-tetradecen-1-ol-acetate that were discovered in BE-II (Alaribe *et al.*, 2020; Fagbemi *et al.*, 2022; Sana *et al.*, 2016). 6-Octadecenoic acid molecule exhibits powerful antioxidant capabilities, including the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, inhibit the peroxidation of lipids, and work in concert with iron-reducing activity (Dong *et al.*, 2019).

Studies have discovered that the fruit of *Smilax excelsa* has a broad spectrum of physiologically active compounds and may be utilized to treat and avoid several illnesses. In the subsequent experiment, the fruit of the *Smilax excelsa* plant had been evaluated for its radical scavenging activity (RSA) using an extract made over the entire physiological ripening process. [Figure 2](#) depicts the RSA study's kinetics, which was analyzed.





**Figure 2.** Kinetics of radical scavenging activity of the extracts

As seen from the kinetics of RSA value of BE-I was higher than BE-II which were  $RSA_{(BE-I)}=62.5\pm0.8\%$  and  $RSA_{(BE-II)}=54.7\pm1.3\%$ . The free radical scavenging activity of BE-I was also considered because the amount of bioactive substances identified in BE-I was greater than the amounts of substances determined in BE-II. As was known from Figure 2, in the kinetics of BE-I extract at the 100th second, the absorption curve from 0.6482 to 0.2148 decreased dramatically. In contrast, the kinetic curve of the BE-II extract changed gradually and slowly from 0.6482 to 0.3568 in intensity until the 300th second. The radical scavenging activity of both extracts was greater than *Citrus sinensis*  $RSA=65.74 \pm 1.98$ , *Cocos nucifera*  $RSA=153.8 \pm 1.98$  %, *Citrus anrantifolia*  $RSA=55.58 \pm 1.00$  in the study of Abdur Rauf *et al.*, equivalent to the amount of dry powder in 3 ml cuvette. But *Citrus limonum*  $RSA=38.51 \pm 1.98$ , *Mangifera indica* fruit  $RSA=44.7\%$ , *Tomato*  $RSA=44.65$ , *Watermelon*  $RSA=40.90$  values got smaller values than the radical scavenging activity of both extracts. The radical scavenging activity of *Malus Sylvestris* (L.) was high compared to  $RSA=56.7\pm10.2$  % BE-I compared to less BE-II. and BE-I extracts (Mustafa *et al.*, 2018; Prakash *et al.*, 2011; Rauf *et al.*, 2014; Shahzad *et al.*, 2014).

#### 4. DISCUSSION and CONCLUSION

BE-I and BE-II, which were derived from the fruit of the *Smilax excelsa* L., have been reported to contain 14 physiologically active chemicals with antibacterial, antifungal, carcinogenic, and antioxidant properties. As can be seen from Table 1 and Table 2, the number of medicinally important substances in the aqueous extract is double less than the number of substances in the alcoholic solution. That is why the antioxidant behavior of these bioextracts varies, with BE-I having a higher activity than BE-II. So that having  $RSA_{(BE-I)}=62.5\pm0.8\%$  and  $RSA_{(BE-II)}=54.7\pm1.3\%$  provides us with the suitability of these bioextracts for therapeutic usage at home condition. The bioextracts produced from the fruit can be employed in the confectionery sector and in the production of alcoholic and non-alcoholic beverages due to the presence of two aromatizing and flavouring compounds. Both extracts have strong antioxidant properties, making them useful for both disease prevention and treatment.

#### Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

#### Authorship Contribution Statement

**Vafa Atayeva:** Methodology Investigation, Finding Materials, Extraction and Writing and antioxidant activity of extractions. **Farhad Azizov:** Interpretation of Results, Writing -original

draft. **Zarbali Khalilov**: Extraction, Activity Experiments. **Nurmammad Mustafayev**: Supervision and Validation. **Hilal Imali**: GC/MS Experiments.

### Orcid

Vafa Atayeva  <https://orcid.org/0000-0002-9886-2500>

Farhad Azizov  <https://orcid.org/0000-0002-9438-066X>

Zarbali Khalilov  <https://orcid.org/0000-0002-8606-4683>

Nurmammad Mustafayev  <https://orcid.org/0000-0002-8493-0429>

Hilal Imali  <https://orcid.org/0000-0002-5251-7867>

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