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Research Article

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Metabolomic Profiling of *Schisandra chinensis* (Turcz.) Baill Leaves and Their Combined Effects with *Malus domestica Borkh* Powder and *Beta vulgaris L.* on Experimental Dental Germ Histogenesis

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Abstract

Schisandra chinensis, a classical natural adaptogen, is utilized in official medicine for its fruits and seeds due to its pharmacological properties, such as adaptogenic, immunostimulating, antioxidant, hepatoprotective, antitumor, and membrane-stabilizing effects. These properties are attributed to a complex of biologically active substances, including lignans, essential oils, polysaccharides, triterpene saponins, vitamins, and macro- and microelements. The study also explores the potential use of plants with various biological components and detoxifying effects, using the example of histogenesis changes in jaw bone tissue and dental rudiments of laboratory animals exposed to ecotoxicants. These ecotoxicants, even in small doses, exhibit strong mutagenic and carcinogenic effects with high cumulative ability and toxicity. The research focuses on identifying marker components with physiological activity in the metabolic profile of *Schisandra chinensis* leaves and examining the impact of its extract, combined with *Malus domestica* and *Beta vulgaris* powders, on dental bud histogenesis. Gas chromatography with a mass-selective detector (GC/MS) was used to analyze three *Schisandra chinensis* leaf extract samples, identifying 34 organic compounds, primarily hydrocarbons, lignans, and terpenoids. Chloroform-based extracts contained the highest number of compounds. The effect of ecotoxicants on rat jaw tissues and dental rudiments was examined in five experimental groups via histological microscopy. Various degrees of dental histogenesis abnormalities and structural changes in dental tissues were observed, including impaired blood circulation, histogenesis of dental rudiments, and viability of ameloblasts and odontoblasts during dental hard tissue formation. Pregnant rats exposed to ecotoxicants and treated with a mixture of *Malus domestica*, *Beta vulgaris* powders, and *Schisandra chinensis* leaf extract gave birth to offspring with fewer dental rudiment and tissue changes.

Keywords: *Schisandra chinensis* (Turcz) Bail; Gas chromatography; Metabolomic profile; Ecotoxicants; *Malus domestica* Borkh powder; *Beta vulgaris* L. powder.

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1. Introduction

In the modern world, the growth of industry, mechanical engineering, and transport has led to the accumulation of ecotoxicants in the environment, negatively impacting human health. For example, dioxins can suppress the immunoenzymometric system, leading to diseases of the blood and hematopoietic system, endocrine disorders, oncological diseases, and congenital deformities. These changes can be inherited and cause polymorphic multilevel disorders of homeostasis, ecological-genetic, neurotoxic, and other consequences [1].

One effective way to detoxify the body is using pectin substances, which exhibit high physiological activity [2-4]. Pectins serve as detoxifiers, removing metabolic products and heavy metals, and have immunostimulating, antitumor, adaptogenic, and other beneficial activities [5]. The main sources of pectins include pomace (a byproduct of fruit juice production), beetroot powder, and citrus peel. Phytochemical studies and biological activity research on the powder of root crops and extracts of *Beta vulgaris* L. have shown that it contains carbohydrates, proteins, fatty acids, and vitamins (in descending order: B2>C>B3>E>B5>B1>B6>K), fiber, phenolic acids (such as caffeic acid, syringic acid, and ferulic acid), flavonoids (such as quercetin, rutin, and myricetin), and betalains (such as betanin, isobetanin, vulgaxanthin I and II) [6-8].

Another way to enhance the body's resistance to ecotoxicants is to increase its endurance through substances that activate overall resistance and attract reserve adaptations. These substances, known as stimulants and adaptogens, mobilize the body's defenses by affecting tissue metabolism, improving efficiency, and aiding adaptation to various stresses [9].

Schisandra chinensis, a Japanese-Manchurian endemic with an intermittent East Asian habitat, is one source of natural adaptogens. The only naturally occurring species in Russia is *Chinese lemongrass* in the Far East. *Schisandra chinensis* is known for its dibenzocyclooctadiene lignans, essential oils, polysaccharides, flavonoids, triterpenoids, and other bioactive substances that stimulate the central nervous, cardiovascular, and respiratory systems; increase blood pressure; and enhance mental and physical performance. Tinctures of the fruits and seeds of *S. chinensis* have hepatoprotective, immunomodulatory, hypoglycemic, and stress-regulating effects and can also enhance hematopoiesis and the body's resistance to intoxication. The qualitative composition of essential oils and the quantitative content of their components are influenced by various factors, including climatic conditions [10-15]. Therefore, studying the lipophilic fraction of *Schisandra chinensis* leaves from the flora of Bashkortostan is relevant for understanding the cultivated plant as a new morphological group of raw materials.

The qualitative composition of essential oils and the quantitative content of their components are determined by various factors, including climatic conditions. Consequently, examining the lipophilic fraction of *Schisandra chinensis* leaves from the flora of Bashkortostan holds significance, particularly in terms of its potential as a cultivated plant and a novel morphological group of raw materials.

This study aims to analyze the metabolomic profile of *Schisandra chinensis* leaves to identify marker components with physiological activity. Additionally, the study aims to evaluate the experimental effects of *Schisandra chinensis* leaf extract, combined with *Malus domestica* and *Beta vulgaris* powders, on the histogenesis of dental germs.

2. Methods and Material

2.1. Materials

The research focused on leaves collected during the peak flowering phase of *Schisandra chinensis*, cultivated in the Republic of Bashkortostan. These specimens were sourced from two distinct locations: the South Ural Botanical Garden Institute, a structural unit of the Federal State Budgetary Scientific Ufa Federal Research Center of the Russian Academy of Sciences, and private gardens within the Ufa district of Bashkortostan (geographical coordinates: 54°49'4" N, 55°34'15" E, at an elevation of 159 meters above sea level).

The raw materials consisted of simple, petiolate leaves characterized by a single leaf blade that was elliptical or obovate, with a pointed tip, wedge-shaped base, subtly serrated edges, and pinnate-marginal venation (**Figure 1**).

Malus domestica powder, composed of 100% malic pectin (Stoing, Russia), is a light cream, odorless powder that forms a viscous solution upon interaction with water. *Beta vulgaris* powder, sourced as a dried, ground, premium-grade material (Sdrava Krasa, Russia), is characterized by its red color and sweet taste.

Figure 1. Leaves of *Schisandra chinensis*

To analyze the distribution of components in extracts from *Schisandra chinensis* leaves via gas chromatography (GC), various solvents were utilized: chloroform (ACS grade, CAS: 67-66-3, TU 2631-105-44493179-07, Russia), petroleum ether (C.P. grade, CAS: 8032-32-4, TU 2631-074-44493179-01, Russia), and hexane (ACS grade, CAS: 110-54-3, TU 2631-158-44493179-13, revision 1, Russia).

2.2. Preparation and characterization of herbal extract

Liquid samples were prepared through maceration, where 1.0 g of crushed raw materials was extracted with various extractants in a 1:10 ratio over 7 days, at a temperature not exceeding 25 °C, in a dark environment shielded from light.

The experimental analysis used an Agilent 8890 GC System gas chromatograph and a quadrupole mass-selective detector (Agilent Technologies 5977B GC/MSD, USA). Chromatograms obtained from the study were decoded for qualitative analysis using the Enhanced Data Analysis – EDUCATION.M software programs and Qualitative Analysis MassHunter Workstation (Version 10.0). Identification of compounds was achieved through comparison with full mass spectrum library data, applying a similarity coefficient threshold of at least 80%. The chromatography conditions were as follows: a capillary HP-5MS column (30 m × 0.25 mm × 0.25 mm) with polydimethylsiloxane (PDMS) as the sorbent was utilized. Helium was the carrier gas at a 1 ml/min flow rate. The column temperature program began at 80 °C, maintained for 2 minutes, followed by a heating rate of 5 °C/min until reaching 290 °C, with a 2-minute hold at the initial temperature and a 10-minute hold at the final temperature. The injector temperature was set at 280 °C, and the sample volume was 1 µl. Detection employed electronic ionization mode (70 eV), with spectra recorded in ion current scanning mode.

2.3. Toxicity Studies

The study on the effects of toxic substances on the histogenesis of rat teeth was conducted at the VGTSPH (All-Union Eye Surgery and Plastic Center) (Muldashevsky Center) in compliance with the "European Convention for the Protection of Vertebrates Used for Experimental or Other Scientific Purposes" (Strasbourg, 1986) and approved by the Ethical Committee of the BSMU, Ministry of Health of the Russian Federation (protocol No. 2 dated February 22, 2022) [16–19]. The experiment utilized white outbred rats weighing 180–250 g, involving a total of 60 animals divided into five groups: one control group and four experimental groups. The animals were housed under standard conditions, including a temperature of 20–23 °C, relative humidity of 50–60%, and an air exchange rate of 10–15 room volumes per hour while being provided with emergency feed. During the estrous cycle, males were paired with females, and fertilization was confirmed by the presence of sperm in vaginal smears (day 1 of pregnancy). Pregnant females were housed in 200-liter standard exposure chambers of the RAMS ETP design.

Toxic substances, specifically gasoline vapors (100 mg/m³) and formaldehyde (0.035 mg/m³), were inhaled at maximum permissible concentrations for atmospheric air in populated areas. These exposures continued around the clock throughout the entirety of pregnancy. The control group was maintained under normal conditions, while the experimental groups were as follows:

1. Exposed to toxic substances alongside Vitrum prenatal supplementation.
2. Exposed under conditions of apple pectin administration.
3. Exposed with aqueous extracts of S. chinensis leaves serving as a membrane-protective component.
4. It is exposed alongside Beta vulgaris powder, a source of B vitamins (including folic acid).
5. Exposed with a combination of *S. chinensis* extract, *Malus domestica*, and *Beta vulgaris* powders.

The aqueous extraction of *S. chinensis* leaves was prepared by dissolving dry extract in water at a 1:10 ratio. Doses were calculated based on scaling for a human adult weighing 70 kg and administered orally per rat body weight (200 g): aqueous *S. chinensis* extract at 0.6 ml, three times daily; *Beta vulgaris* and *Malus domestica* powders dissolved in water (Groups 4 and 2) or aqueous *S. chinensis* extract (Group 5) at doses of 0.075 g/kg and 4.25 mg/kg, respectively.

The study, involving 50 animals (10 per group), concluded with the euthanasia of two-week-old rat pups using inhalation anesthesia. The lower jaws of the animals were excised using pointed scissors and decalcified in a 10% formic acid buffer solution over 30 days [20–22]. Histological sections (5–8 μm) were prepared using a LEICA RM 2145 rotary microtome (LEICA, Germany) and stained by the Van Gieson method [23]. Microscopic analysis was performed with an AXIO IMAGER-Z1 microscope (CARL ZEISS, Germany).

3. Results and Discussion

Chromatograms of liquid samples of *S. chinensis* leaves are shown in **Figures 2, 11, and 15** (Supplementary).

Table 1 shows two parameters of chromatographic peaks of the studied liquid samples of *S. chinensis* leaves: retention time (Ret. time, min) and peak area (Area).

During the qualitative analysis of Sample No. 1, 45 compounds were identified by comparing the sample with mass spectral libraries, as shown in **Figure 2**. Structures 22 were successfully established among these compounds, representing various classes of organic compounds. The structural and group composition of the sample included terpenes such as monoterpenes, sesquiterpenes, and their derivatives (alcohols and ketones), which accounted for 54.55% of the sample. Additionally, hydrocarbons, comprising extreme acyclic and cyclic hydrocarbons and a saturated fatty alcohol known as behenyl alcohol, were present. Lignans, a group of compounds belonging to dibenzocyclooctadiene derivatives, were also identified, specifically Deoxyschisandrin, Schisandrin B, Schisandrin, Wuweizisu C, and Gomisin A, making up 41.67% of the sample, as detailed in **Table 1**.

The analysis revealed that lignans appeared in the chromatogram section with retention times between 20.66 and 22.38 minutes. The chromatographic index "peak area" indicated the dominant substances in the sample, which included Tricosane with a peak area of 23,298,890; Pentacosane with 39,384,635; Behenyl alcohol with 41,518,180; Deoxyschisandrin with 33,583,358; Schisandrin B with 52,630,476; Schisandrin with 149,640,690; Wuweizisu C with 46,597,936; and Gomisin A with 30,450,334. **Figures 3** to 10 provide the mass spectra of these dominant compounds, offering a detailed analysis of their structural characteristics. The MS spectra of the dominant compounds in sample No. 1 are shown in **Figures 1-8S** (Supplementary File).

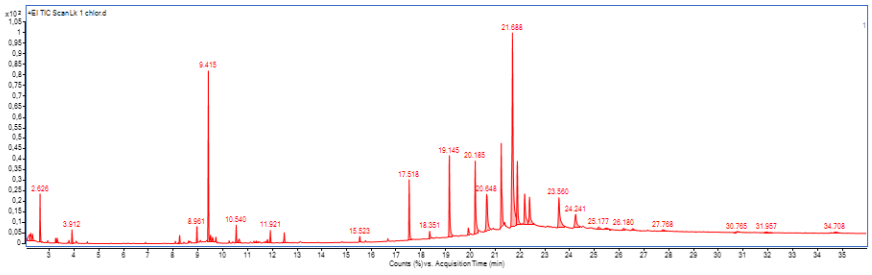

Figure 2. Chromatogram of chloroform extraction (sample No.1) of S. chinensis leaves

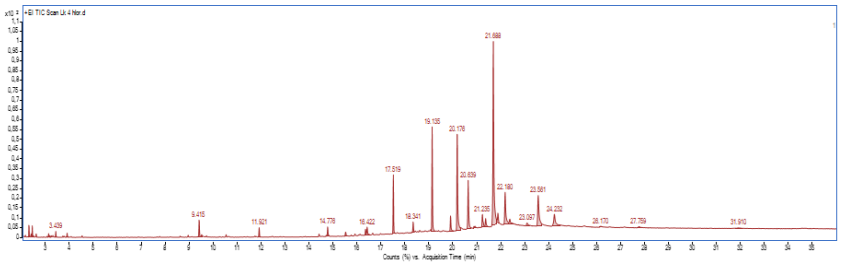

Figure 3. Chromatogram of extraction with petroleum ether (sample No. 2) of S. chinensis leaves

Table 1. Qualitative composition of chloroform extraction of leaves of S. chinensis

№	Name of the compounds	Ret. time, min	Extracts		
			Area, $\times 10^{-6}$	Area, $\times 10^{-6}$	Area, $\times 10^{-6}$
			Sample No. 1 (chloroform), *n=3*	Sample No. 2 (petroleum ether), *n=3*	Sample No. 3 (hexane), *n=3*
1	Bicycle [3.1.1]heptan-6-one, 2-hydroxy-, (1.alpha., 2.beta., 5.alpha.)-	2,305 2,314	13365194	3547249	9033797
2	Cyclohexane, 1-methylene-4-(1-methyl-ethenyl)-	3,316 3,250	4336708	-	2543509
3	(+)-3-Carene	3,903 3,912	51242	1502342	3933198
4	.alfa.-Terpinyl acetate	7,666	-	-	2689548
5	Eugenol	7,770	-	-	2822276
6	1-Methyl-1-ethenyl-2,4-bis (1'-methyl-ethenyl) cyclohexane	8,262	3444872	-	-
7	Isocaryophyllene	8,971	361468	-	3781066
8	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	9,094	3145584	-	-
9	(-)-Germacrene D	9,415	57852882	7037516	28178937
10	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	9,510	4240872	1118026	-
11	.alpha.-Bisabolene	9,585	4631255	-	-
12	(3R, 3aR, 3bR, 4S, 7R, 7aR)-4-Isopropil-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzene-3-ol	10,540	-	1153037	4231384
13	Tau-Cadinol acetat	10,550	6563073	-	-
14	.beta.-Neoclovene	10,644	3180875	-	-
15	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	11,807	4763612	-	-
16	Longifolenaldehyde	12,479	4383337	-	-
17	Hexadecanoic acid	14,432	-	-	4517878
18	Hexadecanoic acid, ethyl ester	14,776	-	5065058	-
19	14-.beta.-H-pregna	15,911	-	-	2266879
20	Linoleic acid ethyl ester	16,356	-	2573912	-
21	9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z)-	16,422	-	3787097	-
22	Tricosane	17,525	23298890	22674143	53834189
23	Tetracosane	18,341 18,351	4667004	4874157	-
24	Pentacosane	19,146	39384635	43970284	95176463
25	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7-dihydroxy-	19,419	-	-	3109826
26	1-Docosanol *Behenyl alcohol*	20,185	41518180	56836191	154539883
27	Deoxyschisandrin	20,658	33583358	-	-
28	1,2,3,13-Tetramethoxy-6,7-dimethyl-5,6,7,8-tetrahydrobenzo[3',4'] cycloocta[1',2':4,5]benzo[1,2-d][1,3] dioxole *Schisandrin B, Gomisin N*	21,239	52630476	9626916	27936069
29	Schisandrin	21,695	149640690	-	382044574
30	Cycloocta (1,2-fi3,4-f)bis (1,3)benzodioxole 6,7,8,9-tetrahydro-1,13-dimethoxy-7,8-dimethyl *Wuweizisu C*	21,888	46597936	10756432	25206021
31	1-Hexacosanol	22,191	-	-	81571133
32	Gomisin A	22,379 22,388	30450334	-	15727852

During the qualitative analysis of petroleum ether extraction of *S.chinensis* (Sample No. 2), 37 compounds were identified through comparison with mass spectral libraries, as presented in **Figure 3**. Among these, the structures of 14 substances representing various classes of organic compounds were determined. The structural and group composition of the sample was as follows: hydrocarbons, including extreme acyclic hydrocarbons, fatty acid esters, and the saturated fatty alcohol Behenyl alcohol accounted for 50.0% of the sample. Terpenes, comprising cyclic monoterpenes, sesquiterpenes, and their derivatives, such as alcohols and ketones, constituted 35.71%. Lignans, represented by Schisandrin B and Cycloocta (1,2-fi3,4-f) bis (1,3)benzodioxole 6,7,8,9-tetrahydro-1,13-dimethoxy-7,8-dimethyl, contributed 14.30% of the total composition, as summarized in **Table 1**. The presence of lignans was detected in the chromatogram within a retention time range of 21.24 to 21.88 minutes, providing a precise window for their identification.

The data of the chromatographic indicator "Area" indicate that in sample No. 2, compounds of a hydrocarbon nature are dominant among the identified substances: Tricosane (22674143), Pentacosane (43970284), Behenyl alcohol (56836191). The MS spectra of the dominant compounds in sample No. 2 are shown in **Figures 9-12S** (Supplementary Files).

During the qualitative analysis of hexan extraction (Sample No. 3), 25 compounds were identified through comparison with mass spectral libraries (**Figure 4**). Among these, the structures of 19 substances belonging to various classes of organic compounds were determined. The primary constituents of the sample included compounds from the terpene group, such as cyclic monoterpenes, sesquiterpenes, and their derivatives (including the aromatic compound phenylpropanoid, specifically Eugenol), which accounted for 42.11% of the total. Compounds from the hydrocarbon group, including marginal hydrocarbons, carboxylic acids, higher fatty alcohols, and their derivatives, constituted 26.32%. Lignans, including Schisandrin B, Schisandrin, Wuweizisu C, and Gomisin A, formed 21.05% of the composition. Additionally, flavonoids (4H-1-Benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7-dihydroxy-) and a steroid compound (14-.beta.-H-pregna) represented 5.26% of the total content, as summarized in **Table 1**. In Sample No. 3, lignans were detected within the chromatogram section, with retention times ranging from 21.24 to 22.39 minutes. Analysis of the chromatographic indicator "Area" revealed that the dominant substances were Tricosane, with a peak area of 53,834,189; Pentacosane, with 95,176,463; Behenyl alcohol, with 154,539,883; and Schisandrin, with 382,044,574. **Figures 13-18S** (Supplementary File) illustrate the MS spectra of these dominant compounds, offering insights into their structural characteristics.

The marker components of Sample No. 3 are predominantly hydrocarbon-based compounds and lignans, specifically Schisandrin. Research on *Schisandra chinensis* has focused extensively on the composition of its essential oils and florentine waters obtained via steam distillation. Studies have concentrated particularly on seed-derived essential oils, including those extracted from waste materials left over after lemongrass juice production. Notably, 36 compounds were identified in seed essential oil, with terpenoids such as β-farnesene, γ-cadinene, panaginsen, α-murolene, β- and α-selinene, trans-kalamenene, and trans-nerolidol being the most prominent. In leaf essential oil, obtained with a 0.51% yield via the GC/MS method, 29 compounds were detected, 21 of which were identified. The primary components included cyclohexylmethyl ether of sulfuric acid, (+)-trans-nerolidol, δ-cadinene, and tridec-(2E)-en-1-ol [24-26].

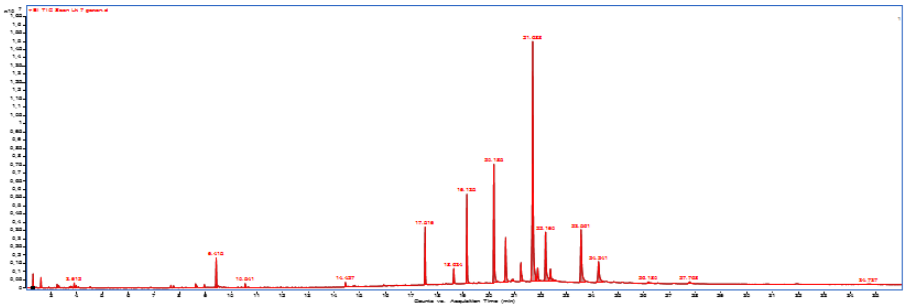

Figure 4. Chromatogram of hexane extraction (sample No. 3) of S. chinensis leaves

Further investigations into *S. chinensis* extracts revealed 76 compounds in ethanol extracts, where the structural and group compositions comprised terpenes, hydrocarbons, their derivatives, and nitrogen-containing compounds. Comparative analysis of phytochemical profiles from lemongrass leaf extracts, using solvents such as chloroform, petroleum ether, and hexane, showed that Sample No. 1 (chloroform extraction) contained 45 compounds, of which 22 were identified. Sample No. 2 (petroleum ether) contained 37 compounds, with 14 identified, and Sample No. 3 (hexane) contained 25 compounds, with 19 identified. Chloroform extracts transferred the highest number of compounds. Among all samples, only eight substances (25.0%) were consistently identified across the three solvents, while the majority were uniquely detected in individual samples.

Using GC/MS analysis, the highest yield of lignans (52.634%) was found in Sample No. 1, with chloroform extracts containing Deoxyschisandrin, Schisandrin B, Schisandrin, Wuweizisu C, and Gomisin A. Significantly, Behenyl alcohol (1-Docosanol) was identified in lemongrass leaves for the first time; this compound has been used in the U.S. as an antiviral agent, particularly for treating herpes, since its approval in 2000. Furthermore, Eugenol, a phenylpropanoid with antibacterial, analgesic, and antioxidant properties, was also discovered for the first time in lemongrass leaves. Other newly identified compounds include the sesquiterpene β-Neoclovene, the flavonoid 4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-6,8-di-β-D-glucopyranosyl-5,7-dihydroxy-, and the tetracyclic triterpenoid Lanosta-8,24-dien-3-ol (lanosterol), as well as a triterpene saponin from the lupeol group, A'-Neogammacer-22(29)-ene.

Experiments were conducted to explore the biological activity of S. chinensis leaves in combination with other plant-derived compounds to evaluate protective effects against ecotoxicants on rat dental tissue. This involved the introduction of *Malus domestica* powder and *Beta vulgaris* powder, individually and in mixtures, demonstrating the potential for integrated plant-based therapeutic applications. The results of the morphological examination of the dental rudiments of baby rats are presented in **Table 2** and **Figures 5 and 6**.

Table 2. The results of morphological studies

Experimental Group	Morphological Pattern
Control Group	Normal histogenesis was observed without any pathological changes.
1st Experimental Group	After exposure to ecotoxicants: Dystrophic changes in the epithelial cells of the mucosa and clusters of segmented leukocytes and lymphocytes. Blood vessels were dilated and blood-filled with extravasation. Odontoblasts showed cytoplasmic dystrophy and necrosis. Dentin and dentine tubules were uneven, with an uneven enamel-dentine line and heterogeneous enamel layers. Osteoblastic cells were absent on bone trabeculae.
2nd Experimental Group	After ecotoxicant exposure with malic pectin: Cytoplasmic changes and nuclear deformation in the mucosal epithelium, with infiltration of neutrophils, lymphocytes, and macrophages, blood vessels showed congestion and vasodilatation. The enamel-dentine line was uneven, with immature secondary enamel appearing as "foamy" islets and lacking primary and final enamel layers.
3rd Experimental Group	After ecotoxicant exposure with a membrane protector from *S. chinensis*: Changes in the epithelial basement membrane and submucosal base. Blood vessels were dilated and blood-filled, with some dystrophic alterations in odontoblasts. Dentin and prismatic enamel were uneven, with deformed ameloblasts and ulcerated zones in the final enamel. The swelling was noted between odontoblasts.
4th Experimental Group	After ecotoxicant exposure with *Beta vulgaris* powder, Pulp showed dilated blood vessels with polymorphism in pulp stromal cells. Disruption of enamel formation stages was evident. Connective tissue showed swelling, with significant edema around dilated blood vessels.
5th Experimental Group	Most dental germs exhibited normal structural elements after ecotoxicant exposure with combined use of malic pectin, S. chinensis membrane protector, and Beta vulgaris powder. Pulp was free of edema, odontoblasts were well-differentiated, and layers of predentin, dentin, and prismatic enamel were smooth and well-formed. Periodontal tissues developed normally, with minimal dystrophic changes in mucosal epithelial cells limited to mild cytoplasmic swelling without nuclear destruction.

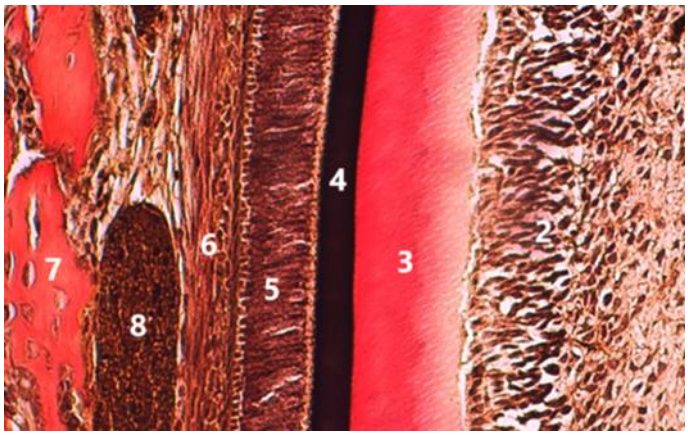

Figure 5. The structure of a rat's dental bud after ecotoxicant poisoning:
1 - pulp; 2 - odontoblasts; 3 - dentin; 4 - enamel; 5 - ameloblasts; 6 - periodontium; 7 - alveolar bone; 8 - blood-filled vessels (x 100)

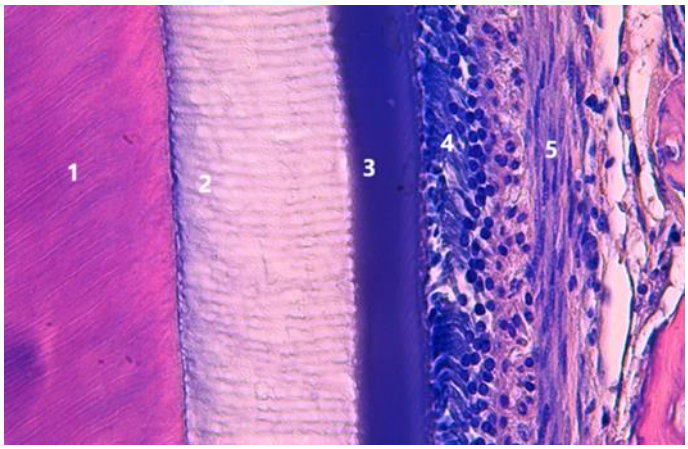

Figure 6. The structure of the dental bud of a rat of the fifth experimental group:
1 - dentin; 2 - secondary prismatic enamel; 3 - terminal enamel; 4 - enameloblasts; 5 - periodontium; 6 - alveolar bone. Hematoxylin and eosin staining (x200)

Experimental studies have demonstrated that significant disruptions in dental histogenesis were observed in the first experimental group, which involved ecotoxicant poisoning, along with structural alterations in the alveolar tissues and dystrophic changes in the oral mucosa. The rats in the second experimental group exhibited mild abnormalities in dental histogenesis and alveolar tissue structure. Additionally, dystrophic changes were identified in the gingival mucosa, accompanied by impairments in enamel formation within the dental germs.

In the third experimental group, histological preparations of the jaws indicated that the gingival mucosa exhibited minimal pathomorphological changes. However, dystrophic changes were detected in isolated epithelial cells. Abnormalities in the structure of dental germs were present, including irregularities in the development of enamel and dentin. In the fourth experimental group, pronounced morphological changes were observed in the immature teeth of baby rats. This group demonstrated severe deformation and destruction of odontoblasts and a disruption of the sequential stages in enamel layer formation.

Studies by Kamilov H.P. et al. (2019) demonstrated that prenatal exposure to toxic substances, including pesticides, sulfur dioxide, and nitrogen, impaired fetal and postnatal development. These effects are most pronounced when the combined toxicity of pesticides and dioxins is present. The study further revealed that intrauterine intoxication significantly disrupts the differentiation of dental tissues and jawbones in the fetus, leading to delayed eruption and abnormalities in tooth development during the postpartum period [27].

Analysis of histological preparations from experimental group 5 exhibited nearly normal histogenesis at the late stages of dental development. Most immature teeth displayed typical structural elements, and the gingival mucosa of the rats lacked pronounced pathomorphological changes. This outcome can be attributed to the beneficial effects of polysaccharides derived from *S. chinensis*, *Malus domestica*, and *Beta vulgaris*. These polysaccharides have been shown to promote health by reducing cholesterol, insulin, and glucose levels in the blood. Additionally, they exert immunostimulatory effects, such as increasing gamma interferon levels in mesenteric lymph node lymphocytes, boosting immunoglobulin (E, A, G, and M) levels, and stimulating lysosomal activity in peripheral phagocytes. They also exhibit significant anti-inflammatory properties [28].

The membrane-protective action of *S. chinensis* is explained by its ability to inactivate free radicals within cells [29]. *Schisandra* leaf extracts interfere with various aspects of free radical formation, including generating reactive oxygen species and suppressing lipid peroxidation caused by free radicals, which are prevalent in biological systems. Moreover, the B-vitamin complex present in *Beta vulgaris* powder contributes positively to hematopoiesis, normalizes metabolic processes, and

enhances immune function. Folates, in particular, play a critical role in nucleotide synthesis and the normal growth of all body cells [30].

4. Conclusion:

In conclusion, this study represents a significant advancement in understanding *S. chinensis* leaf extracts and their applications. Through a detailed comparative analysis using GC/MS with various solvents (chloroform, hexane, and petroleum ether), the study identified 59 organic compounds, primarily hydrocarbons, lignans, terpenoids, and flavonoids. The results highlight the necessity of employing diverse solvents for a comprehensive phytochemical profile of *S. chinensis* extracts.

Additionally, the research demonstrated that administering a combination of *S. chinensis* leaf extract and Malus domestica and Beta vulgaris powders in pregnant rats exposed to ecotoxicants positively influenced their offspring's dental and parotid development. This group exhibited improved development of dental germs and parotid tissues compared to others, which showed disruptions in blood circulation, the histogenesis of dental germs, and the functionality of ameloblasts and odontoblasts. These findings open pathways for further research into such natural compound combinations' protective and restorative potential.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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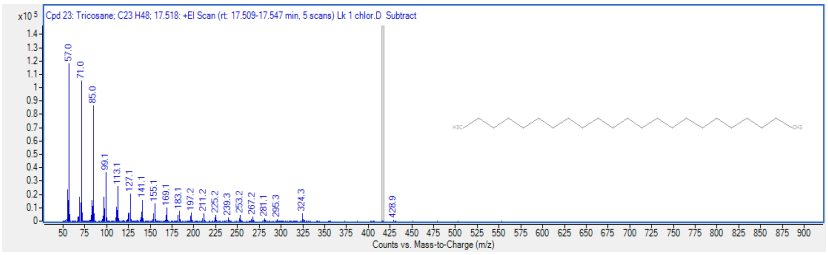

Figure 1S. MS spectrum of Tricosane sample No. 1 of *S. chinensis* leaves

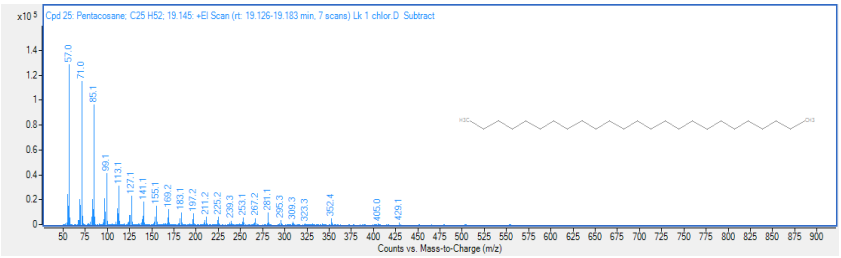

Figure 2S. MS spectrum of Pentacosane sample No. 1 of *S. chinensis* leaves

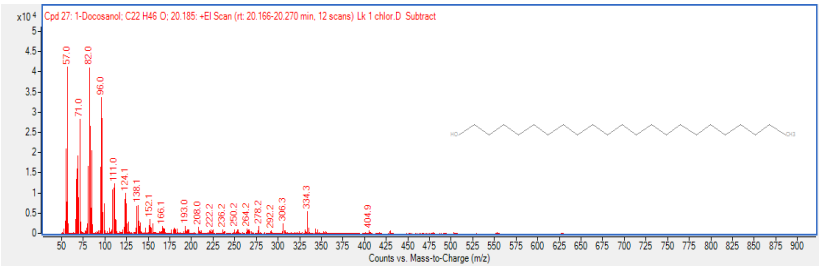

Figure 3S. MS spectrum of Behenyl alcohol sample No. 1 of *S. chinensis* leaves

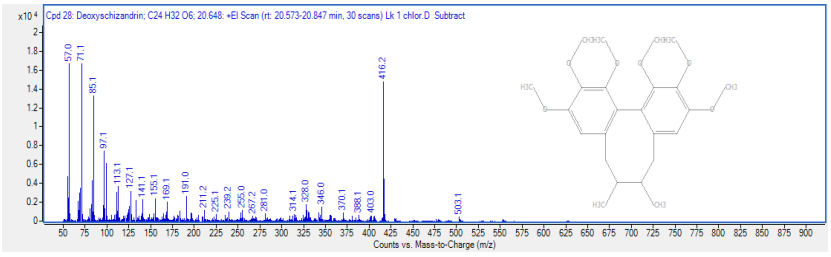

Figure 4S. MS spectrum of Deoxyschisandrin sample No. 1 of *S. chinensis* leaves

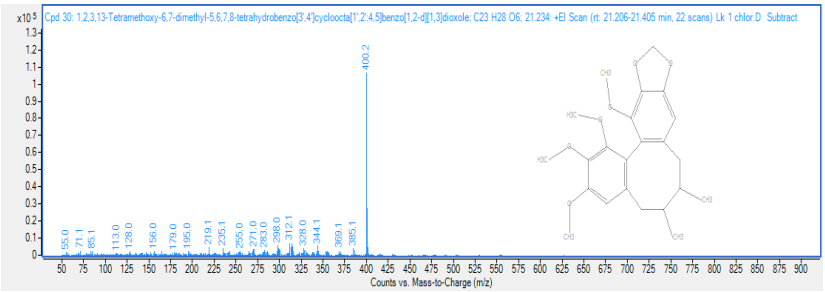

Figure 5S. MS spectrum of Schisandrin in sample No. 1 of *S. chinensis* leaves

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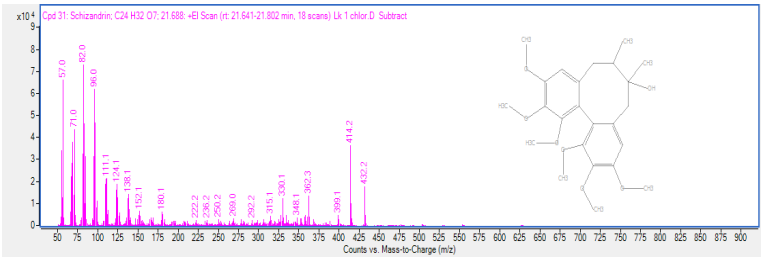

Figure 6S. MS spectrum of Schisandrin sample No. 1 of *S. chinensis* leaves

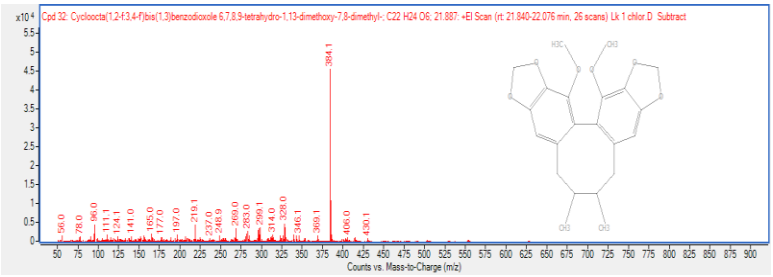

Figure 7S. MS spectrum of Wuweizisu C sample No. 1 of *S. chinensis* leaves

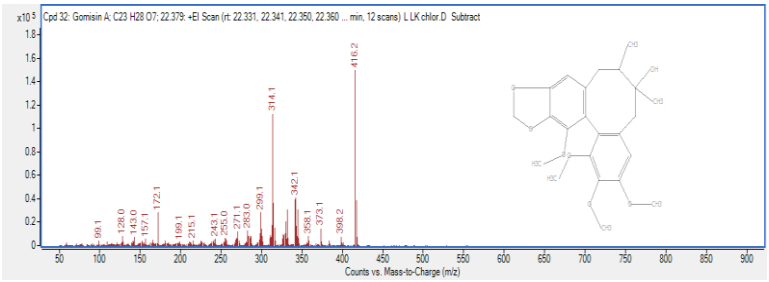

Figure 8S. MS spectrum of Gomisin A sample No. 1 of *S. chinensis* leaves

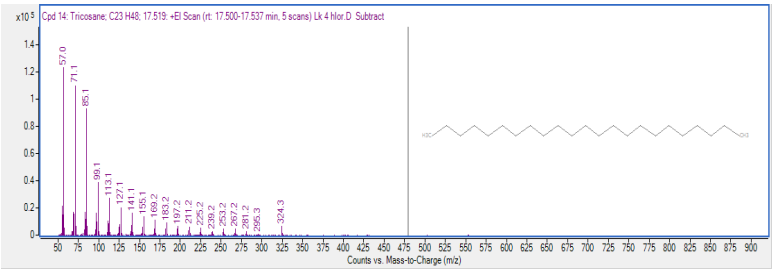

Figure 9S. MS spectrum of Tricosane sample No. 2 of *S. chinensis* leaves

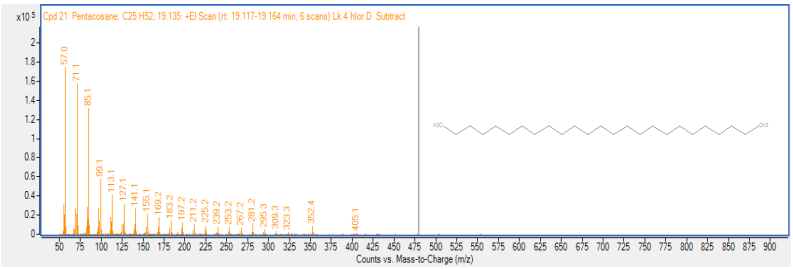

Figure 10S. MS spectrum of Pentacosane sample No. 2 of *S. chinensis* leaves

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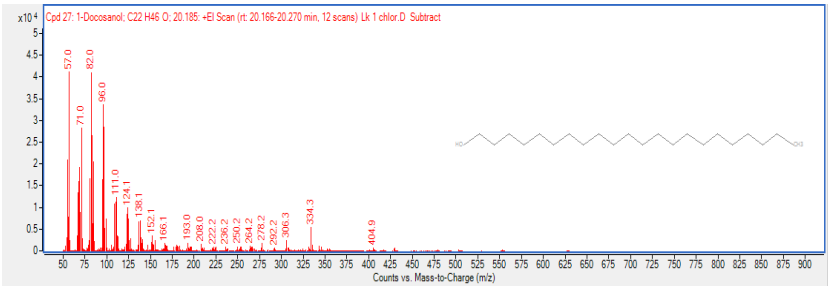

Figure 11S. MS spectrum of Behenyl alcohol sample No. 2 of *S. chinensis* leaves

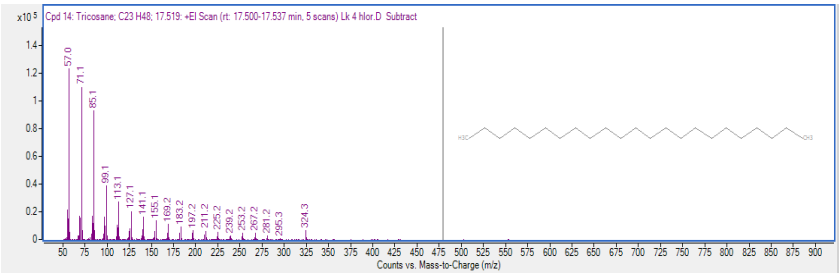

Figure 12S. MS spectrum of Tricosane sample No. 2 of *S. chinensis* leaves

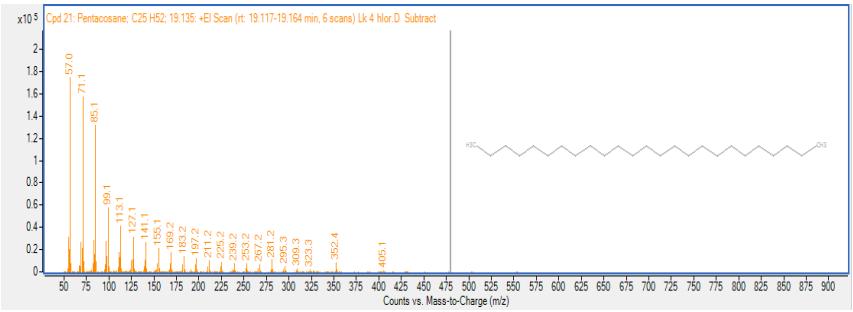

Figure 13S. MS spectrum of Pentacosane sample No. 2 of *S. chinensis* leaves

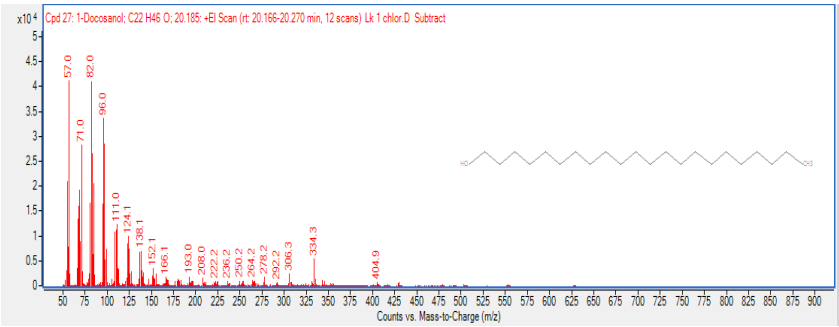

Figure 14S. MS spectrum of Behenyl alcohol sample No. 2 of *S. chinensis* leaves

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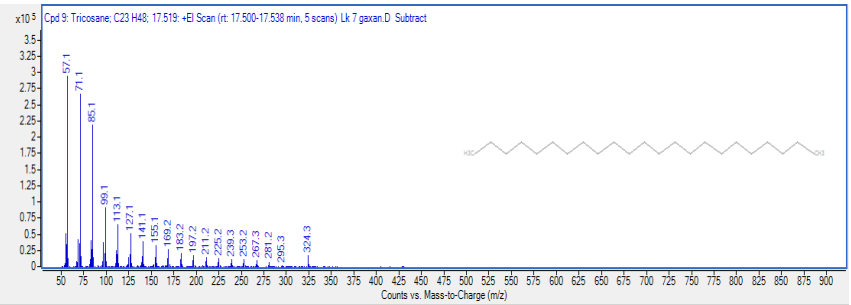

Figure 15S. MS spectrum of Tricosane sample No. 3 of *S. chinensis* leaves

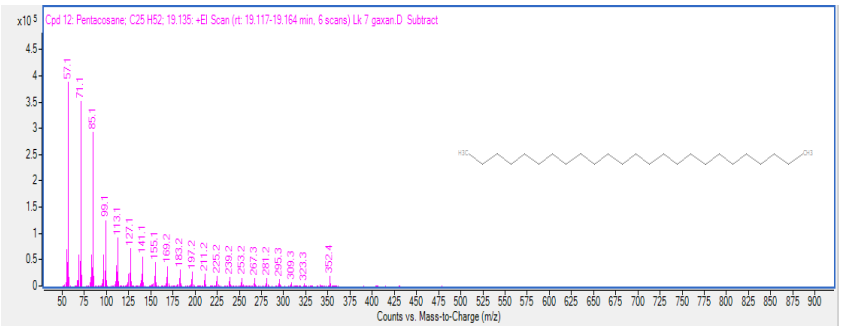

Figure 16S. MS spectrum of Pentacosane sample No. 3 of *S. chinensis* leaves

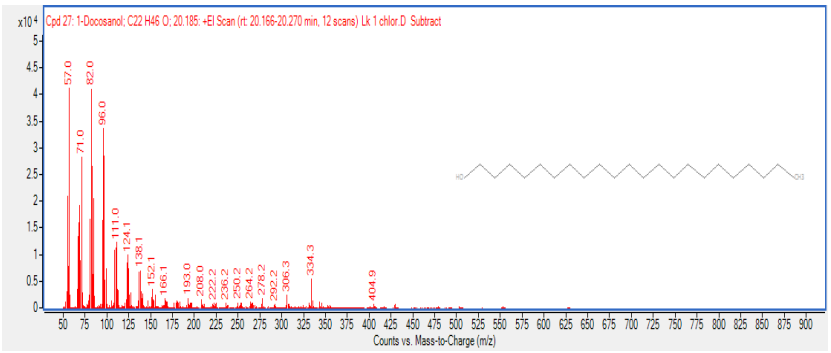

Figure 17S. MS spectrum of Behenyl alcohol sample No. 3 of *S. chinensis* leaves

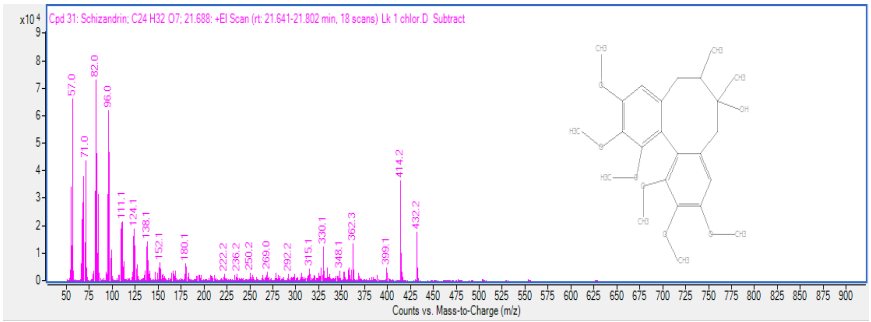

Figure 18S. MS spectrum of Schisandrin sample No. 3 of *S. chinensis* leaves