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THE INFLUENCE OF RHIZOBACTERIA ON SOME SECONDARY METABOLITES OF FERULA: A REVIEW

M.A. Nematova, S.S. Murodova

Ferula L. plants are of great importance in a number of fields such as agriculture, veterinary, medicine and food industry. Medicinal properties of Ferula L. species are explained by the variety of secondary metabolites they produce, in which the activity of root rhizobacteria occupies a special place.

This article presents the research-based analysis of some biological properties, agrotechnology, secondary metabolites (ferulin A,D; diversolid A,D,F,G; kuhistanicaol A,B,C,D,G; kuhistanol (A-H); fucanefuromarin (A-G); fucanemarin A,B; fesumtuorin (A-H); ferulagol A,B; pallidon (A-F); sinkiangenorin D and others) of the species of genus Ferula L., the influence of the microbial communities (new species belonging to the genera Porphyrobacter, Paracoccus and Amycolatopsis, as well as Actinobacteria, Acidobacteria, Proteobacteria, Gemmatimonadet and Bacteroides) living in the roots of Ferula L. on the synthesis of some secondary metabolites of the plant, based on scientific sources.

Keywords: *microbiology; Ferula; endophytic bacterial community; rhizobacteria; secondary metabolites; terpenoid; kuhferin; ferulin; kufestrol; review*

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Introduction

Species of the genus *Ferula* L., which belongs to the family *Apiaceae*, are a source of biologically active substances, coumarins, ethers, flavonoids, terpenoids, and other natural compounds, which are the research object of many native and foreign scholars all over the world. By now, 180-185 species of *Ferula* L. have been identified on Earth, in particular, 106 species in CIS countries, 105 species in Central Asia and 48 species in Uzbekistan [8], 19 species in Jizzakh region [21].

The glue (resine, tar) obtained from the roots of many species belonging to the genus Ferula L. (*F. kuhistanica F.foetidissima*, *F.kokanica*, *F.foetida, F.kopetdagensis, F.soongarica F.persika, F.sumbul, F.kapsica F.badrakema, F.diversivittata, F.varia F.karatavika, F. gummoza, F.karelini*) has long been called Kovrak, Kinna, Ushturghoz, Sumbul Sassiq Kovrak, Galbanum, Spagen, and other names. This special glue was used in the treatment of spleen, kidney, stomach, liver diseases, as well as gynecological diseases; furthermore, it was applied as an appetite suppressant, joint pain reliever, diuretic medicine, against asthma, sclerosis, bronchitis, jaundice, diabetics, whooping cough, urinary and kidney pain, even to stop bleeding [62].

In recent years, due to the unplanned use of these medicinal plant resources, their reserves have decreased, and some species are under threat of complete disappearance. There is a high demand in world markets for the resin extracted from the root of the plant. It takes an average of 5 years for one seedling to produce resin.

Using the biotechnological potential of rhizobacteria, which accelerates the growth and development of plants, is one of the urgent problems in the cultivation of ferula.

In this research work, we aimed to study analytically the biological properties, agrotechnology, secondary metabolites, and microbial communities of the Ferula. L genus and the effect of rhizobacteria on plant terpenoids based on scientific articles published in authoritative databases.

Particular biological properties of the species belonging to the genus *Ferula*. L

Ferula L. occupies a special place in the flora of the desert and mountain regions of Uzbekistan. The systematics, distribution, carpology, biology, ecology, reserves, chemical and pharmacological properties of the species of this genus were initially studied by Korovin E.P. (1947, 1951, 1959), Nikonov G.K. (1971), Nishanbaeva H. (1972), Pimenov M.G. (1977, 1983), Vinogradova V.M. (1990), Meliboev S. (1985), Saidkhodzhaev A.I. (1985), Nikolayeva. M (1948) Kurmukov A.G, Akhmedkhodzhaeva Kh.S. (1994) Safina LK. (2015) Rahmankulov. U. (1999; 2016) and many other scientists.

In recent years, scientific researches based on a number of modern approaches have been carried out in the study of the species belonging to the genus *Ferula*. L.

Avalboyev O.N.studied and developed measures for their protection of the species belonging to the genus *Ferula* L., which are widespread in the Western Pamir-Aloy range and have economic value and important medicinal properties. Furthermore, recommendations on methods of propagation of *Ferula foetida* species were developed and introduced into processing practice. The current states of populations of 10 monocarp and polycarp species belonging to the genus *Ferula* L. found in the Western Pamir-Aloy range (*F. samarkandica* Korovin, *F. kuhistanica* Korovin, *F. diversivittata* Regel et Schmalh., *F. helenae* Rakhmankulov et Melibaev, *F. foetida* (Bunge) Regel, *F. kokanica* Regel et Schmalh., *F. penninervis* Regel et Schmalh., *F. dshizakensis* Korovin, *F. ovina* (Boiss.) Boiss., *F. angrenii* Korovin) were studied, and their distributional GAT maps were developed [8].

Muqumuv I.U.et al. found the presence of 0.6% mixture of complex ethers in the above-ground part of *Ferula kuhistanica* at the beginning of vegetation, the main of which are ferutinin, ferutin and teferin, while the total amount of ethers gradually increases to 2% during the fruiting period as the plants grow [40].

Ahmedov E.T.et al. studied the composition of fatty acids in the resin of *Ferula assa-foetida L*. According to the research results, there are various types of fats in plant resine, and the main content of saturated fatty acids is 61.1%, and the amount of unsaturated fatty acids is 38.9%. Based on the results, it was noted that there is a possibility of wide use of these medicinal plant resources on an industrial scale [3]

Turginov O.T. and others analyzed endemics of the flora of Uzbekistan on the basis of samples stored in the literature and herbarium funds and found out that there are 22 species belonging to 13 genus of the *Apiaceae* family, which are endemic to the flora of Uzbekistan. Among them, 5 species belong to the *Ferula* L. genus, and they found that they make up 8.47% of the species of the genus in the flora. Analyzing the index of endemism among other Asian countries, it was found that 23 species are distributed in the flora of Turkey, 13 of which have the status of endemic, and endemics make up 56.52% of the species of the genus in the flora. In the flora of the Islamic Republic of Iran, there are 32 species of the genus, 30 of which are endemic to the region. This made up 93.75% of the species of the general category. There are 26 species distributed in the Chinese flora, 7 species are endemic and make up a quarter of the total species in the flora. Based on the results of the analysis, it was determined that the regions of Asia and the Mediterranean correspond to the centers of origin of the species of the group [69].

Khalkuzieva. M.A. studied the biology, growth, development, biomorphology of *F. tadshikorum and F. foetida* species, and analyzed the characteristic features of the embryonic (*se*- seeds), virginal (*r* - grass, *j* - juvenile, *im* - immature, *v* - virginal) and generative periods in the ontogeny of these species. As a result of the research, it was found that *F. tadshikorum* and *F. foetida* species do not have complete ontogenesis periods, and at the same time, senile period is not observed in these species, flowering and seeding periods are very short [26].

Yusupov O.Sh. analyzed the chemical composition of *Ferula assafoetida*, which is common in Kyzylkum region, during the growing season, and scientifically substantiated the negative effect of the sap obtained from the root and stem of the plant and the grain on the body of korakul sheep. During the study, the sap of the stem and root of *Ferula assafoetida* was given to korakul sheep in 100 ml for 14 days, from the 8th day of the experiment, 150 grams of its grain was given to each sheep for 60 days, and the clinical signs of poisoning appeared on the 28th day of the experiment. Also, information about the occurrence of changes in some morphological, biochemical and immunological indicators in the blood of sheep is given [76].

Abdulmyanova L.I and others isolated 10 communities of endophytic fungi from the *Ferula foetida* plant growing in the southwestern part of Kyzylkum. When the samples were tested for antimicrobial activity and the content of growth stimulants, it was found that the antimicrobial properties of *Fusarium sambucinum* - FF59S are the highest among the selected endophytes. The growth-restricted zone for *Staphylococcus aureus* is similar to that of the antibiotic gentamicin and is found to be greater than 15 mm. When the growth promoting activity was studied, all tested endophytes were found to contain indole acetic and gibberellic acid, with the highest titers of indole acetic and gibberellic acid being *Alternaria sp*.- FF63L - 280 mcg/ml and 40 mcg/ml and *Fusarium sambucinum*-FF59S - 300 mcg/ml and 50 mcg/ml, respectively[1].

Najimitdinova N.N. studied the chemical composition of two representatives (*Ferula tatarica* Fish. ex Spreng va *Ferula soongorica* Pall. ex Spreng) of the genus *Ferula. L* and analyzed their pharmacological properties. Furtermore, a method of obtaining a drug with estrogen properties has been developed [43].

Representatives of the genus *Ferula* L have attracted the attention of many world scientists due to their high medicinal properties and wide range of pharmaceutical possibilities.

In particular, N. Hadi et al. analyzed the growth of zygotic embryos of *Ferula gummosa* Boiss. *in vitro* and noted that zygotic embryos of galbanum are not suitable for germination *in vitro*.

However, it was found that embryos formed better callus in nutrient medium containing 0,3 mg l-1 GA_3, 2 mg l-1 BA and 10 mg l-1 NAA, ¼ MS (¼ macro elements MS) [15].

Mostafavi K.studied the allelopathic effect of the aqueous extract prepared from the seeds, roots and stems of *F. gummosa* Boiss at three different concentrations on the germination and seedling growth of three weeds (common amaranth, purslane and wild barley). All components significantly inhibited seed germination rate, percentage, hypocotyl and root length at the highest concentration when applied before germination. When *F. gummosa* extract was applied after germination, it had an effect on root and stem length, wet and dry weight of plants. Based on the results, it was determined that *F. gummosa* is a source of allelochemical substances, and these substances cause dormancy of *F. gummosa* seeds [40].

Hassani B. et al. for the first time succeeded in obtaining plant seedlings from *Ferula assa-foetid*a through direct and indirect somatic embryogenesis. About 40-50% of the obtained somatic embryos turned into full-fledged plants. After 12 weeks, undamaged seedlings without callus phase were obtained in direct somatic embryogenesis in a hormone-free medium. During embryo induction, it was observed that the hypocotyl parts are the most on the entire seedling surface. More than 50% of cotyledon embryos formed plant seedlings and rooted [16].

Koorki Z. and others studied the high insecticidal activity of essential oils obtained from *Ferula assa-foetida* L., toxicity and anti-activity of essential oils obtained from *Achillea wilhelmsii L. and Ferula assa-foetida L.* against two-day-old *Aphis gossypii* larvae. Mortality rates were found to increase with increasing concentrations of essential oils and exposure time from 12 to 24 hours. When the composition of the essential oil was analyzed, (E)-1-propenyl sec-butyl disulfide (43.16%) and (Z)-1-propenyl sec-butyl disulfide (27.45%) were found in *Ferula assa-foetida L.*, camphor (29.03%) and 1,8-Cineol (12.86%) were found in *Achillea wilhelmsii L.*. These compounds have insecticidal activity against various insects, and it was noted that they can be an important resource in the fight against *A. gossypii* [30].

Popova O.A. and others extracted a dry extract of *Ferula assa-foetida* L. Resin. Based on the extract, a thymogar preparation was developed. Ethers were qualitatively determined by vanillin solution in conc. H_2SO_4 (1%), polysaccharides were determined using conc. H_2SO_4 and phenol, coumarins were determined by TLC using the umbelliferone standard. Also, the content of thymogar, total phenolic compounds and flavonoids was quantitatively determined. Based on the results, an anti-diabetic formula was developed [52].

Azizov D.Z.and others extrated different groups of polysaccharides from the roots of *Ferula tenuisecta*, and compositions were created from their monosaccharides. It was determined that the extracted pectin substances have an esterification level of 56.25%. The activity of water-soluble polysaccharides against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli and Klebsiella oxitoca 1*) and spore-forming bacteria (*Bacillus subtilis*) has been determined [9].

Rakhmanov H.S.determined the ontomorphogenesis of *Ferula tadshikorum*, an endemic of Southern Tajikistan, the ecological conditions of the environment where the species is distributed, the morphological characteristics of the species and the ontogenetic structure of its senopopulations. Phenology and reproductive biology were studied under natural growth conditions, and natural reserves of medicinal raw materials were identified. Also, the total area of ferulas distributed in the territory of Southern Tajikistan and their productivity have been determined [56].

Furthermore, the use of *Ferula* L. species in the national economy, systematics, ethers, tar, secondary metabolites and chemical composition of volatile compounds, cultivation and tar collection methods, based on scientific sources were deeply analyzed by Yaqoob U. et al. (2016), Upadhyay P.K. (2017), Sun L. et al. (2013), Mohammadhosseini M. (2019), Sood R. (2020), Shah N.C. (2019), Salam N (2019), Salehi M. (2019).

Agrotechnology of the species of the genus *Ferula* L.

During 2016-2019, Rakhmankulov U et al. studied the morphology and germination dynamics of *F. foetida* and *F. tadshikorum* seeds collected from different regions of the Republic in room conditions. According to the results of the research, it was found that the latent period of plants is more than 4 years, *F. tadshikorum* germinated 89% in the newly harvested year (2019), and 25% of the seeds stored for 4 years germinated. The germination rate of *F. foetida* was 59-12 percent, respectively. It is recommended to use freshly collected or second-year seeds for planting ferulas [55].

Avalbaev O.N. and others studied the germination of the seeds of some species of the genus *Ferula* L. found in the West Pamir-Oloy at different temperatures, by keeping them for different periods. The seeds of the studied 10 species of *Ferula* L. were collected in summer, and the seeds were stored at room temperature for 3-4 months. After that, it was grown in a Petri dish at a temperature of 0-+4°C. It has been found that harvesting freshly collected seeds at a temperature of 0 - +4 °C is also effective. Saving seeds for two years and harvesting them under snow also gave good results, and it was recommended to use this method for planting seeds of monocarpic species that do not bear fruit every year [7].

In 2018, Hamrayeva D.T. and others conducted a research on *Ferula tadshikorum* by sowing them in two variants and phenological observation was carried out in the experimental plot of the Tashkent Botanical Garden. According to the results of the experiment, the growth rate of *Ferula tadshikorum*, like most introduced plants, was accelerated. Compared to the natural environment, *Ferula tadshikorum* grown in plantations has positive economic efficiency due to the shortening of its life cycle and the possibility of obtaining raw materials from underground bodies in a short period of time. Also, the optimal planting period for growing *Ferula tadshikorum* has been determined [27].

According to the recommendation of Khojimatov O.K, since spring comes much earlier in the southern regions of the republic, it is advisable to sow the seeds of *F. tadshikorum* in the autumn-winter period, from the beginning of November to the first ten days of December. In this case, the seeds undergo a period of natural stratification for 80-90 days. The researchers noted that in the conditions of Tashkent, after the air temperature reaches + 5°C, it is necessary to start planting, and the seedlings appear at the end of the first ten days of February or at the end of February and the beginning of March. It is also recommended to sow *F. tadshikorum* seeds in natural conditions at a depth of 2-3 cm on soils with various grass cover and on eroded, crushed and rocky slopes at a depth of 1.0-2 cm [29].

Khalkuziyeva M.A., U. Rakhmonkulov U. studied the germination of *F. tadshikorum* in soils with different composition, under natural and moisture-preserved conditions. In December, 100 plant seeds were sown separately in sandy, gray and potash-rich soils at a depth of 0.5-1.0 cm in 2 different options. According to the results, in the 1st variant of the experiment, the fertility of *F. tadshikorum* seeds in soil enriched with potassium was significantly higher than in other soils, and the fertility of seeds planted in 62% sandy soil was equal to 32%. In the 2nd version of the experiment, the seeds were harvested by providing water. The germination rate of seeds in soil enriched with potash and other organic fertilizers was 80%, and the germination rate of seeds planted in sandy soil was 26%. When the seeds of *F. tadshikorum* and *F. foetida* were planted and grown in the same soil conditions for three years in two variants, it was found that their vegetation lasts longer in agrotechnically treated and irrigated environment compared to plants grown in natural conditions [25].

Allayarov M.U. et al. developed a recommendation on the establishment of *Ferula* L. plantations. According to the research, it is recommended to sow 1 ripened and cleaned *Ferula* L. seeds in depth of 5-10 cm, and at a time of drought,in the depth of 3.5 cm, and cover the top with soil. To increase productivity, it is recommended to use 200 - 300 kg of potassium sulfate per hectare. It was determined that 5-6 kg of seeds are spent on 1 hectare of land, and on average 2500 seedlings can be grown, and after 7 years, on average, 100-125 kg of sap can be obtained from each seedling. At the same time, it is recommended to plow the field, clean it from weeds and stones, and sow selected seeds in autumn and early spring [4].

Various experiments were conducted in order to improve the fertility of the seeds of *Ferula* L. species. Cold storage of seeds followed by treatment with 6-benzylaminopurine (BAP) [48] or using cytokinin[16] kinetin[75] have been found to increase the germination of seeds. Washing seeds for 14 days and cooling at 5° C [42] has also been found to give effective results. Providing plants with mineral fertilizers during the growing season is another factor that increases productivity. In the first year, it is recommended to use 15-20 t/ha of natural fertilizers, and chemical fertilizers of 20 g N, 18 g P_2O_5 and 25 g K_2O per seedling. This amount was gradually increased, and from the tenth year, 200 g N, 180 g P_2O_5 and 200 g K_2O were applied per plant.[65,5].

During the period of germination, seedlings have a high need for moisture, and during the remaining periods, excess moisture in the ground can damage the roots. Reza et al (2015) recommended watering the mats once a week. The resin yield, vegetation and viability of *Asafoetida* are also related to the number of irrigations. Therefore, it is recommended that the relative humidity of the soil be 40-75%. It has been found that weeding twice a year until the seedlings are four to five years old has a positive effect on increasing its productivity [65].

Secondary metabolites of plants belonging to the genus *Ferula L.*

Many species of *Ferula* L. are known for their resine and secondary metabolites with different phytochemical properties. The derivatives of many ferula species *F. latisecta* Rech. f. & Aell., *F. gummosa* Boiss., *F. assa-fetida* L., F. *sinkiangensis* KM Shen., F. *fukanensis* K.M.Shen., F. *kuhistanica* Korovin, F. *penninervis* Rgl. et Schmalh., F. *ferulaeoides* (Steud.) Korovin, F. *badrakema* Koso.- Pol., F. *sinaica* Boiss., F. *persica* Willd. and others are the main raw materials of the pharmaceutical industry. All over the world, many scientific researchers have been conducted on the secondary metabolites produced by these plants. The main derivatives of *Ferula* species are gummosin, farnesiferol A, farnesiferol C and badrakemon, umbelliprenin [28], ferulin A, D; diversolid A,D,F,G; kuhistanicaol A,B,C,D,G; kuhistanol (A-H); fucanefuromarin (A-G); fucanemarin A,B; fesumtuorin (A-H); ferulagol A,B; pallidone (A-F); sinkiangenorin D [39] and a number of other chemical compounds.

According to Swargiary G. et al., secondary metabolites are chemicals produced by plants that are not directly important for plant growth. These compounds are usually produced by fungi, bacteria and other microorganisms. Although secondary metabolites are found in almost all plants, they are seasonal. Because they are synthesized only in selected special cells and at a certain stage of growth. Secondary metabolites are divided into certain groups (Table 1).

The presence of various secondary metabolites is one of the main criteria that ensure the medicinal properties of plants [67].

Table 1.

Classification of secondary metabolites [67]

Categories	Species	Examples
Terpenes	Monoterpenes	Farnesol
	Sesquiterpenes	Limonene
	Diterpenes	Taxol
	Triterpenes	Digitogenin
	Tetraterpenoids	Carotene
	Sterols	Spinasterol
Phenols	Lignan	Lignin
	Tannins	Gallotanin
	Flavonoids	Anthocyanin
	Coumarins	Umbelliferone
Nitrogen and sulfur containing compounds	Alkaloids	Nicotine
	Atropine	
	Glucosinolates	Sinigrin

Chen B. et al. isolated 13 known daucan ethers along with seven daucane-type sesquiterpenes named kuhistanicaol A-G from methanol extracts of air-dried roots and stems of *Ferula kuhistanica* (Figure 2). Their structures are studied on the basis of spectroscopic evidence and the results of chemical reactions [14].

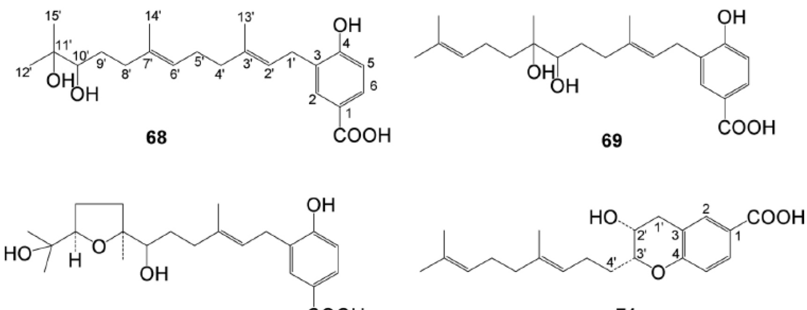


Figure 1. The molecular structures of kuhistanols A-D (68-71) of *F. kuhistanica* Korovin [14,39]

Chen B. et al. extracted four new prenylated benzoic acid derivatives, kuhistanols A-D, and one known compound, 3-farnesyl-p-hydroxybenzoic acid from the roots of *Ferula kuhistanica* (Fig. 1,3). Their structure was studied on the basis of chemical and spectral evidence. Kuhistanol D significantly counteracted the cytokine production of human peripheral mononuclear cells stimulated with lipopolysaccharides [13].

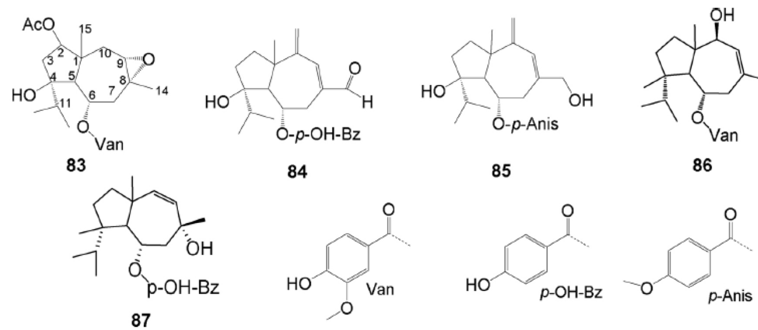


Figure 2. The molecular structures of five daucane-type sesquiterpenes (83-87) characterized from methanol extract (stem and root) of *F. kuhistanica* Korovin [14,39]

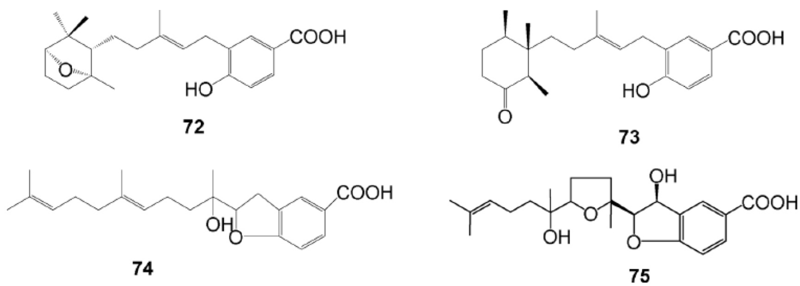
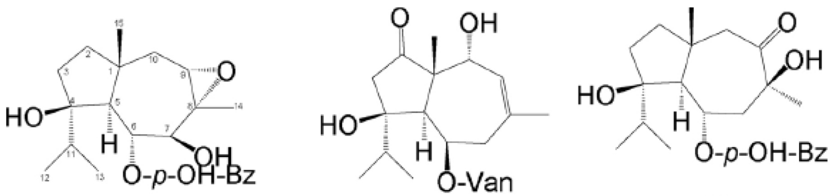


Figure 3. The molecular structures of farnesyl hydroxybenzoic acid derivatives (72-75) in the MeOH extract of *F. kuhistanica* Korovin roots [13,39]

Tamemoto K. et al. extracted three daucan ethers: kuhistanicaol H, I and J along with nine other known compounds from ethyl acetate extracts of air-dried fruits of *Ferula kuhistanica* (Figure 4). Their structures were studied on the basis of spectroscopic evidence. The antibacterial activity of these isolated compounds and previously isolated compounds from roots and stems of *F. kuhistanica* was tested. Some of them were toxic to gram-positive bacteria, including methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* [68].

Khazaei A. et al evaluated the effect of different treatment with gibberellic acid (GA_3) on the germination of *Ferula pseudalliacea* seeds. According to the results, treatment with 500 micromolar GA_3 and 8 weeks of cooling was more effective. Also, explants from young leaves, stems, cotyledons and embryos of *F. pseudalliacea* were inoculated with five strains of *Agrobacterium rhizogenes*

ATCC 15834, 1724, A4, LB9402 and Ar318. Although the transformation efficiency of strain ATCC 15834 (4%) was higher than that of 1724 (2%), root hairs were formed using strains ATCC 15824 and 1724 only in 10-12-day-old embryonic explants. The maximum frequency of root hair changes was found to be 10 minutes (25%), 20 minutes (20%) and 30 minutes (5%). In addition, the transformation percentage was 6% at 24 hours, 22% at 48 hours, and 29% at 72 hours, depending on the time of inoculation. Transgenic root hairs were confirmed by PCR amplification of the *rol*B gene. Root hair networks were found to produce higher biomass in half B5 (Hamborg) medium than in half MS (Murashige and Skoog 1962), and it was found that the number and dry biomass of root hairs from strain ATCC 15834 was higher than strain 1724. When cross-analyzing transgenic root hairs and natural root extracts, all transgenic root hair branches were found to produce farnesiferol B[28].



94 95 96

Figure 4. The main daucane esters (94-96) of *F. kuhistanica* Korovin, which were extracted from the EtOAc extract (dried fruits) [68,39]

For the first time, Asilbekova D.T. and others studied the essential oil and lipids of the leaves of *Ferula kuhistanica* Korovin using GC-MS, and it was determined that the composition of the essential oil contains 51 components (mainly 72.9% α-pinene, 99.8% in total). The composition of the essential oil was analyzed enantioselectively and the enantiomeric excess of (+/–)-α-pinene (61.9/38.1), (+/–)-β-pinene (28.6/71.4), (+/–)-sabinene (13.2/86.8) and (+/–)-limonene (82.7/17.3) was determined. Hexadeca-7Z, 10Z, 13Z-trienoic acid in lipids confirmed that *F. kuhistanica* belongs to the 16:3 plant group [6].

Khalilova E.Kh. and others analyzed the secondary metabolites in the aerial part of *Ferula foetida* by GC-MS, and found out that the main components in *F. foetida* sap are 2,3,4,5-tetramethylthiophene and 2-ethylthiopyridine. Furthermore, 1,3-dimethyltrisulfan, 1,8-cineole (eucalyptol), S-methylmethanethiosulfonate, 2,3,4,5-tetramethylthiophene, (+)-trans-chrysantenyl acetate, (+)-calarin, bulnesol, etc. were determined in the content of extract [24].

By using spectroscopic methods, Iranshahi M. et al. revealed that methanolic extract of *Ferula persica* dried roots contains four sesquiterpene coumarin glycosides, persicaosides A-D, and two known phytosterol glucosides, sitosterol 3-O-β-glucoside and stigmasterol 3-O-β-glucoside [20].

Saidkhojayev A.I. and Nikonov G.K.obtained ferutidin ($C_{23}H_{32}O_4$) by extracting silicagelda from the roots of *F. kuhistanica* Eug. Kor. collected during the growing season and ferutinin ($C_{20}H_{30}O_4$) by washing the roots with petroleum ether-ethyl acetate (3:1) [59].

Babekov A.U.and others studied the essential oils of sesquiterpene alcohols from the roots of *Ferula kuhistanica* Eug. Kor. collected from the Haydarkon mining area of Fergana region and determined teferin, tenuferin, ferutin, ferutinin, tenuferidine and tenuferinins by extracting on a silico-gel column. They also identified a new substance characteristic of terpenoid esters with the composition $C_{30}H_{42}O_8$ and named it kuhferin. (Figure 5) [10].

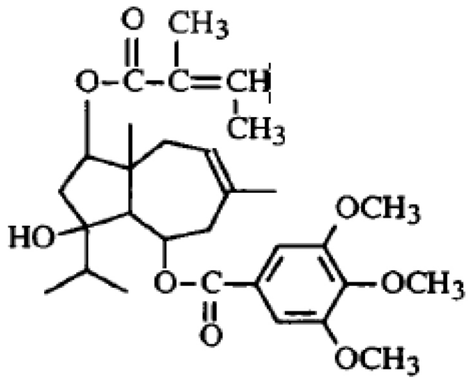


Figure 5. Kuhferin [10]

Khalifaev P.D. and others obtained essential oils by hydrodistillation from three samples of *Ferula kuhistanica* collected from two different regions in the central part of Tajikistan. In the experiments, a total of 77 compounds were identified, making up 95.8-99.9% of the total oil content. The essential oils of *F. kuhistanica* roots contain monoterpene hydrocarbons and it was determined that α-pinene (57.7-70.6%), β-pinene (8.2-27.1%), β-phellandrene (0.1-7.2%) and myrcene (1.5-2%) prevails [23].

Nazrullaev S.S. et al. compared the estrogenic and reproductive activity of a total terpenoid alcohol ether compound extracted from the aerial parts of *Ferula kuhistanica Korov* (kufestrol) and the roots of *Ferula tenuifida* (tefestrol).

Research results have shown that kufestrol is close to tefestrol. Kufestrol is mainly a mixture of three compounds ferutinin (75-80%), ferutin (10-15%) and akicheinin (7-10%), while tefestrol is composed of ferutinin (95%), tenuferidine (2-3%) and fertidine (residues). Kufestrol and tefestrol have been found to have slightly less estrogenic effects than diethylstilbestrol and ethinyl estradiol [44].

Hoseinpour F. and Lorigooini Z. reported about secondary metabolites such as coumarin, sulfur-preserving compounds, stigmasterol, sitosterol, monoterpene and sesquiterpene, as well as anti-Alzheimer, anti-inflammatory, antibacterial properties of *F. persica* [18]

Xing Y. et al. found that sesquiterpene coumarins in *Ferula sinkiangensis* have anti-neuroinflammatory effects [73].

Microbial communities in the roots of the species belonging to the genues *Ferula* L.

Researches based on a number of biotechnological approaches have been carried out in order to plant and grow, to increase the fertility and productivity of *Ferula* L. species, which has gained industrial importance in recent years.

Researching the activity of microbial communities living on the root surface of the plant and in the soil layer is one of the promising areas of biotechnology.

According to Lucy M. et al., PGPR increases plant germination rate, root growth, yield, magnesium content, leaf size, chlorophyll content, protein content, nitrogen content, drought tolerance, shoot and root mass, and hydraulic activity. It slows down the aging of leaves. It also increases the plant's resistance to diseases [37].

Pseudomonas, Bacillus, Burkholderia, Stenotrophomonas, Micrococcus, Pantoea, Microbacterium are the most common endophytes in the plant rhizosphere [63].

Information on the study of microbial communities in the rhizosphere of *Ferula* L. plants was originally published by Zhu J. et al., *Ferula fukanensis* K.M. Shen. and *Ferula sinkiangensis* K.M. Shen [77,78].

Liu Y.H. and others analyzed the diversity of cultured bacteria isolated from the medicinal plant *Ferula songorica* collected from Hebukesaier, Xinjiang. A total of 170 endophytic bacteria belonging to three phyla, 15 orders, 20 families and 27 genera were isolated and characterized by 16S rRNA gene sequencing. The order *Actinobacteria* was the main group of endophytic microbes isolated from *F. songorica* plant (107 isolates). It was found that endophytes are more present in the root tissues of the plant compared to the leaf and stem tissues. Of these endophytic strains, 88% can grow in nitrogen-free environments, 19% are phosphate soluble, and 26 and 40% are protease and cellulose producing, respectively [36].

Liu Y. and others analyzed the endophytic bacterial community of *Ferula sinkiangensis* for plant growth promoting properties, and a total of 125 endophytic bacteria belonging to 3 phyla, 13 orders, 23 families and 29 genera were identified based on 16S rRNA gene sequence data. Among the different isolates, three strains isolated from roots identified potentially new species of the genera *Porphyrobacter, Paracoccus* and *Amycolatopsis*. In this study, 79.4% and 57.1% of the total isolates were reported to produce indole-3-acetic acid and siderophore, respectively. 40.6% of the strains inhibited the growth of the fungal pathogen *Alternaria alternate*, 17.2% and 20.2% of the strains showed properties against *Verticillium dahlia* 991 and *V. dahlia* 7 [35].

Wang X. et al. conducted a research on 4 types of ferulas growing in Xinjiang region of China [*F. gracilis* (Ledeb.) Ledeb., *F.syreitschikowii* K.-Pol., *F. lehmannii* Boiss., and *F. ferulaeoides* (Steud.) Korov.] in order to determine the richness and diversity of rhizosphere bacterial communities. According to the results of the analysis, the soil depth affected the structure of the bacterial community, some rhizosphere bacteria were sensitive to the soil depth and the medicinal value of the plant, and as the soil depth decreased, the medicinal value of *Ferula* species and the number of the unique rhizosphere bacteria increased [72].

Zhang T. et al. studied the diversity of rhizosphere bacteria of the desert ephemeral plant *Ferula sinkiangensis*, and determined that it contains *Actinobacteria* (25.5%), *Acidobacteria* (16.9%), *Proteobacteria* (16.6%), *Gemmatimonades (11.5%))* and *Bacteroides* (5.8%). They also studied the trends in the diversity of the bacterial community on mountain slopes and soil depths at different altitudes [78].

Effect of rhizobacteria on plant terpenoids

According to Zehra A. et al., aromatic and medicinal plants contain biologically active secondary metabolites such as flavonoids, alkaloids, steroids, sesquiterpenes, terpenes, saponins, phenolic substances and diterpenes. These secondary metabolites have anti-helminthic, anti-inflammatory, anti-malarial, analgesic, anti-microbial, anti-fungal, anti-arthritic, anti-oxidant, anti-hypertensive, anti-diabetic, anti-cancer, anti-histaminic and cardio-protective properties. Secondary metabolites play an important role in the adaptation of plants to changes in the external environment and stress. Abiotic and biotic stress that can occur in plants affects the production of secondary metabolites. Abiotic (heat, cold, salinity, drought) stress has been found to induce the production of reactive oxygen species in plant cells [77].

Khadka D. et al. suggested that plants can produce secondary metabolites using plant growth-promoting rhizobacteria (PGPR). PGPR in the rhizosphere enhances plant development by synthesizing the cytokine, gibberellin hormones, and indole-3-acetic acid; enriches the soil with mineral nitrogen, and also fights disease-causing microorganisms to protect plants. Based on scientific sources, it is proved by researchers that treatment of plants with PGPR increases the synthesis of secondary metabolites in them, which have the properties of treating diabetes [22].

Cappellari L.R. et al analyzed the effects of plant growth-promoting rhizobacteria (PGPR) and exogenous application of salicylic acid or methyl jasmonate on plant total phenolic content and monoterpenes in *Mentha x piperita* plants. The results showed an increase in total phenolic content associated with increased phenylalanine ammonia lyase enzyme activity. Also, the use of methyl jasmonate in combination with PGPR at different concentrations resulted in an increase in total phenolic content. A synergistic effect was observed. The concentration of the main monoterpene present in peppermint essential oil was also significantly increased when salicylic acid or methyl jasmonate was applied. It has been proved that methyl jasmonate or salicylic acid together with PGPR is effective in improving the production of secondary metabolites from *M. piperita* [11].

Ibrahim A. et al. conducted field tests and laboratory experiments to evaluate the effectiveness of treatment with rhizobacteria in controlling *Meloidogyne incognita* in eggplant. In the experiment, the total phenol content of the plants was significantly different between the control (0,3 g/kg) and RB5 (3.4 g/kg, treated with rhizobacteria RB). Peroxidase content was determined in control (0.6 g/kg) and treated plants (2.54 g/kg), and the content of peroxidase in different rhizobacterial treated plants had different results. The amount of terpenoids in the control (0.29 g/kg) and RB1 (0.54 g/kg) RB2 (0.83 g/kg), RB3 (1 g/kg) and RB4 (1.02 g/kg). The highest amount of terpenoids was observed in RB5 (1.23 g/kg). The results confirmed that this process is significantly more effective than the use of ascorbic acid and polyphenol oxidase enzyme. It has also been proven that treatment with rhizobacteria activates the defense properties of eggplant plants faster against *M. incognita* [19].

Carlson R. et al. treated *Sorghum bicolor* (L.) Moench under greenhouse conditions with rhizobacterial isolates (*Bacillus* and *Pseudomonas*) and found that there was a significant difference in the characteristics of resistance to rust between the control groups. In *S. bicolor* plants inoculated with rhizobacteria, the main metabolic changes that ensure stress tolerance in severe drought conditions are (1) increase in antioxidant content; (2) high control of root development by indole acetic acid, gibberellic acid, and cytokinin hormones; (3) activation of early systemic tolerance signaling hormones brassinolides, salicylic acid and jasmonic acid and signaling molecules by psychosine and sphingosine; (4) production of glutamic acid, proline and choline osmolytes; (5) production of epicuticular wax docosanoic acid; (6) reduction of ethylene as a result of 1-aminocyclopropane-1-carboxylate deaminase activity [12].

Based on behavioral, chemical, and gene-transcription studies, Pangesti N. et al. investigated the importance of the rhizobacterium *Pseudomonas fluorescens* WCS417r in indirect plant defense against the leaf-feeding insect *Mamestra brassicae*. As a result of research, it has been found that beneficial microbes in the rhizosphere change the physiological state of plants and increase their protection against insects directly and indirectly [49].

Ahmadzadeh M. et al analyzed the effect of probiotic bacteria on the amount of alkaloids in *Catharanthus roseus* and the expression of genes of terpenoid indole alkaloids (vinblastine, vincristine) with strong anticancer activity by biosynthesis. The individual and collective effects of *A. brasilense* Ab-101 and *P. fluorescens* 169 strains were evaluated. Furthermore, the content of vinblastine and vincristine was determined in the roots of *C. roseus*, and compared to the control, it was observed that it increased sharply to 174 and 589 mcg/g, respectively. According to molecular analysis, *P. fluorescens* increased the expression of the CrPRX gene by 47.9 times compared to the control. Anological results were observed in *A. brasilense* strains. Based on the evidence obtained, it has been proven that there is a correlation between transcription and metabolic outcomes [2].

Conclusions

Plants belonging to the genus *Ferula* .L, the family *Apiaceae* are important in a number of fields such as agriculture, medicine, food industry and veterinary medicine [62,56], to study the biological, ecological, healing properties of the representatives of the genus, and to protect the endangered species are one of today's urgent issues.

Planting seeds in the late autumn, weeding seedlings, watering once a week, using natural and mineral fertilizers in the cultivation of *Ferula* L. helps to increase productivity [4,57].

As a result of components farnesiferol A. farnesiferol C gummosin, and badrakemon, umbelliprenin ferulin A, D; kuhistanicaol A,B,C,D,G; diversolid A,D,F,G; kuhistanol (A-H); fucanefuromarin (A-G); fesumtuorin (A-H); fucanemarin A,B; ferulagol A,B; zinciangenorin D; pallidon (A-F) [39,28] and a number of other secondary metabolites creates the potential of using *Ferula* L. species on a large scale in the pharmaceutical industry.

New species of *Porphyrobacter, Paracoccus* and *Amycolatopsis* genera, as well as *Actinobacteria, Acidobacteria, Proteobacteria, Gemmatimonadet* and *Bacteroides* were extracted from the root rhizosphere of representatives of *Ferula* L. [35,78]. These bacteria stimulate the growth and flexibility of *Ferula* L.

Plant growth-stimulating rhizobacteria have a positive effect on the production of secondary metabolites, activate the protective properties of plants, increase a number of properties such as resistance to stress in drought conditions and protection against insects [12]. Also, the use of rhizobacteria is effective in increasing the amount of terpenoids and alkaloids in medicinal plants [22].

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