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Study of the Component Structure of the Metabolites of Bacteria *Nocardiopsis umidischolae* in the Search for Eco-Friendly Plant Protection Agents

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Abstract—The component structure of the suspension and extracts (water-alcohol, methanol, and hexane) of isolates of *Nocardiopsis umidischolae* nos. 2 and 18 strains having high rates of aphicidal and acaricidal activities and lacking phytotoxicity was studied using the methods of qualitative tests and thin layer chromatography (TLC). The composition of the water-alcohol extracts from the actinomycete strains was analyzed by high performance liquid chromatography (HPLC). The HPLC analysis of the metabolites of *N. umidischolae* no. 2 bacteria revealed the presence of isocitric, acetic, fumaric, malic, lactic, and citric acids. The metabolites of *N. umidischolae* no. 18 culture contained isocitric, acetic, fumaric, and lactic acids.

Keywords: actinomycetes, metabolites, isolates, suspension, extract, eluent, thin layer chromatography, high performance liquid chromatography, metabolites

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INTRODUCTION

A wide variety of products of secondary metabolism of actinomycetes, found in nature, demonstrate huge chemical diversity. They are represented by aliphatic, carbocyclic, and heterocyclic, nitrogen-, oxygen-, and sulfur-containing compounds whose molecules contain various functional groups: ether, ester, carboxy, hydroxy, epoxy, amino, and nitro groups [1–3]. Most of the isolated secondary metabolites that are synthesized by actinomycetes have antibiotic (antimicrobial, antiviral) activity [4–7, 8–11]. Also, some of them possess a different kind of biological activity; these are enzyme inhibitors, herbicides, and insecticides, which find plant growth applications [12–14].

EXPERIMENTAL

In this study we used 3 day suspension (10^9 CFU/mL) and 5 variants of extracts [water-alcohol in three modifications (80% : 20%, 50% : 50%, 20% : 80%), methanol, and hexane] from isolates of *N. umidischolae* nos. 2 and 18 strains, which showed high insectoacaricidal activity and phytostimulating properties.

Isolates were identified by 16S DNA sequencing in the Departmental Collection of Beneficial Microorganisms for Agricultural Purposes (All-Russia Research Institute of Agricultural Microbiology, St. Petersburg, Pushkin).

For preparing extracts, the crude biomass was dried at 40°C for 7 days. The sample dried to a constant weight was ground in a mortar to a particle size of 1–2 mm. Water-alcohol extracts were prepared by pouring 1 mg of dry biomass of the strain suspension with 1 mL of (a) 80% : 20%, (b) 50% : 50%, and (c) 20% : 80% ethanol:distilled water solution .

For obtaining methanol extracts, 1 mg of the dry biomass was poured with 1 mL of methanol, and after 1 h the resultant extracts were centrifuged. The supernatant of the extract was taken for analysis.

To prepare hexane extracts, 250 mL of the suspension was extracted with 5 mL of hexane. Extraction with nonpolar solvents (hexane, petroleum ether, gasoline, etc.) is known to afford recovery of a large group of substances with biological activity from microorganisms. The resultant hexane extracts were stored in a refrigerator.

The component structure of the metabolites of the bacterial cultures studied was analyzed by means of qualitative tests and thin layer chromatography on Sorbfil PTSKh-AF-A-UF plates (10×15 cm). The water-alcohol extracts of the actinomycetes were studied by high performance liquid chromatography.

The chemical composition of the suspension and bacterial extracts was evaluated by means of qualitative tests for the presence flavonoids, alkaloids, glycosides, and saponins [24].

Presence of flavonoids was checked by the reaction with ammonia solution via adding 3–5 drops of ammonia solution to 1 mL of the filtrate. Flavones, flavonols, and flavanones gave yellow coloration, turning into orange or red on heating; chalcones and aurons gave orange or red coloraton, and anthocyanins, blue or purple coloration.

The Wagner–Bouchard precipitation reaction was used as a test for alkaloids, to which end 3–5 drops of Wagner–Bouchard reagent were added to a test tube with 2 mL of the extract. Also, Marquis test was carried out for this substance group by adding 3–5 drops of Marquis reagent (sulfuric acid + 40% formalin in a 25 : 1 ratio) to 2 mL of the extract, which resulted in formation of a brown precipitate of alkaloids.

Glycosides were detected using the Keller–Kiliani test specific for the carbohydrate moiety of the molecule. Into one test tube, 1–2 mL of glacial acetic acid was poured, whereupon 1 mL of the studied extract was added; into the second test tube, 1–2 mL of concentrated sulfuric acid was poured. From the first test tube the solution was carefully poured along the wall into the second test tube. In the presence of glycosides, a brown or dark brown ring appeared on the boundary of the two layers, and the top layer gradually painted in blue or blue-green color.

Saponins were identified by the persistence foam test (a modification of the physical method). The extract (aqueous) was shaken in a test tube for 15 s. Persistent appearance of foam lasting for at least fifteen min indicated possible presence of saponins.

Analytical TLC was performed with the use of silica gel-coated glass plates (Macherey–Nagel) in the ascending mode; visualization followed the techniques of staining in a chamber with crystalline iodine and UV radiation exposure.

Identification of the components was based on the retention factor R_f which is defined as the ratio of the distance from the starting line to the center of the substance zone to that from the starting line to the front line.

Thirty-eight systems of solvents (eluting solvents) with different polarities were used in the chromatographic procedure : acetone, benzene, benzene : methanol (1 : 1), benzene : methanol : acetic acid (1 : 1 : 1), butanol, butanol : acetic acid (1 : 1), butanol : acetic acid : water (1 : 1 : 1), hexane, hexane : ethyl acetate (1 : 1), methanol, acetic acid, chloroform, chloroform : acetic acid (9 : 1), ethyl acetate, ethanol : hexane : ethyl acetate (1 : 1 : 1), ethanol : acetic acid (1 : 1), ethyl acetate : methanol : water (1 : 1 : 1), ethyl acetate : tetrachloroethane : water (1 : 1 : 3), ethyl acetate : methanol (2 : 1), water : sodium citrate : citric acid (2 : 1 : 5), butanol : water : ethanol (4 : 2 : 1), butanol : methanol (1 : 1), ethanol : water (4 : 1), ethanol : water (2 : 8), ethanol : water (5 : 5), ethanol : water (8 : 2), methanol : benzene : chloroform (4 : 2 : 1), chloroform : ethyl acetate (1 : 2), benzene : methanol (3 : 1), propanol : ethyl acetate : water (5 : 1 : 3), chloroform : methanol (1 : 1), propanol : acetic acid : water (3 : 3 : 2), acetonitrile, acetonitrile : water (2 : 2), isopropanol, ethanol : water (7 : 3), chloroform : methanol (9 : 1), chloroform : methanol (95 : 5).

Organic acids in the water-alcohol extracts obtained from the dry bacterial biomass were determined by HPLC using a Waters–Alliance 2695 separation module with a Waters 2996 diode array detector at a wavelength of 220 nm (anion exchange columns, suppression conductivity detection) at the State Research Institute of Genetics and Selection of Industrial Microorganisms, Kurchatov Institute National Research Center.

Mathematical analysis of the findings of this study was carried out using Excel and BioStat 2008 software. Statistical analysis was performed by calculating the arithmetic means (M) and their standard errors (m).

RESULTS AND DISCUSSION

The prepared extracts and suspensions were qualitatively analyzed for the presence of the main substance groups by using tests for glycosides, saponins, alkaloids, and flavonoids as constituents of the metabolites of the bacteria studied. Substances detected in the two isolates

Table 1. Results of the qualitative tests with *N. umidischolae* isolates nos. 2 and 18

Reaction	Substance group	Expected result	Isolate no.	Actual result[a]					
				Water-alcohol extract (20% : 80%)	Water-alcohol extract (50% : 50%)	Water-alcohol extract (80% : 20%)	Hexane extract	Methanol extract	Suspension
Reaction with ammonia solution	Flavonoids	Yellow coloration turning into red or orange on heating, blue or purple coloration	2	+	+	+	–	+	+
			18	+	+	+	–	+	+
Persistent foam test	Saponins	Persistent appearance of foam lasting for at least 15 min	2	–	–	–	–	–	–
			18	–	–	–	–	–	–
Wagner-Bouchard precipitation reaction	Alkaloids	Precipitate formation	2	–	+	+	+	+	–
			18	+	–	–	+	–	–
Keller-Kiliani test	Glycosides	Appearance of brown or dark brown ring on the border of two layers, gradual appearance of a blue-green layer on top of the colored strip	2	+	+	+	–	+	+
			18	+	+	+	–	–	+

[a] (–) Expected result not achieved; (+) expected result achieved.

proved to be similar (Table 1). Saponins were not revealed in the suspensions and extracts of both *N. umidischolae* isolates, which evidences their absence or occurrence in insignificant amounts.

Flavonoids were detected in all the studied samples of the isolates except for the hexane extract. Test for glycosides gave identical results. Alkaloids were revealed in all the samples analyzed except for the suspensions of the isolates, in which this substance group was present in very small amounts or was totally absent. In the case of *N. umidischolae* isolate no. 18 alkaloids were detected in the water-alcohol (20%:80%) and hexane extracts only. The hexane extracts contained alkaloids solely.

The presence of glycosides was confirmed by formation of an emerald ring in all the test tubes with the water-alcohol extracts and suspensions, as well as in those with the methanol extract from *N. umidischolae* strain no. 2.

The component structure of the metabolites of *N. umidischolae* nos. 2 and 18 isolates was evaluated by thin layer chromatography with the use of 456 Sorbfil plates; the elution time was 15 to 110 min.

The plates were placed in the TLC chambers, dried, and irradiated with the light generated by a mercury-quartz lamp in the Testing Laboratory of the Astrakhan oblast Rossel'khoztsentr Branch.

Table 2. Identification of the chromatographic zones in the chromatograms of *N. umidischolae* no. 2 isolate

Run no.	Eluent	Suspension $R_f \pm 0.02$	Water-alcohol extract $R_f \pm 0.02$			Hexane extract $R_f \pm 0.02$	Methanol extract $R_f \pm 0.02$
			20%:80%	50%:50%	80%:20%		
1	Acetone	0.0705	0.0349	0.0562	0.0751	–	–
2	Benzene	–	–	–	–	–	–
3	Benzene-methanol (1 : 1)	0.1149	–	–	0.0602	–	0.0779
4	Benzene : methanol : acetic acid (1 : 1 : 1)	0.3049 0.1929 0.0976	–	–	–	–	–
5	Butanol	–	0.7176	–	0.6706	–	–
6	Butanol : acetic acid (1 : 1)	0.7407	–	–	–	–	0.9438
7	Butanol : acetic acid : water (1 : 1 : 1)	0.7222	–	–	–	–	–
8	Hexane	–	–	–	–	–	–
9	Hexane : ethyl acetate (1 : 1)	–	–	–	–	–	–
10	Methanol	0.1666	–	–	–	–	–
11	Acetic acid	0.1369 0.2328 0.3698	–	–	–	–	–
12	Chloroform	–	–	–	–	–	–
13	Chloroform : acetic acid (1 : 1)	0.0344	–	–	–	–	–
14	Ethyl acetate	–	–	–	–	–	–
15	Ethanol : hexane : ethyl acetate (1 : 1 : 1)	–	–	–	–	–	–
16	Ethanol : acetic acid (1 : 1)	0.3975	–	–	–	–	–
17	Ethyl acetate : methanol : water (1 : 1 : 1)	–	0.1460	–	0.1460	–	–
18	Ethyl acetate : tetrachloroethane : water (1 : 1 : 3)	–	–	–	–	–	–
19	Ethyl acetate : methanol (2 : 1)	–	–	–	–	–	–
20	Water : sodium citrate : citric acid (2 : 1 : 5)	0.8556	–	–	–	–	–
21	Butanol : water : ethanol (4 : 2 : 1)	0.2816	0.2561	0.3256	0.2439	–	0.2250
22	Butanol : methanol (1 : 1)	0.6538	0.6538	0.6364	0.6875	–	–
23	Ethanol : water (4 : 1)	0.7682	–	–	–	–	
24	Ethanol : water (2 : 8)	0.8505	0.8667	–	0.8667	–	–
25	Ethanol : water (5 : 5)	0.9333	–	–	–	–	0.8720
26	Ethanol : water (8 : 2)	0.7037	–	–	–	–	–
27	Methanol : benzene : chloroform (4 : 2 : 1)	–	–	–	–	–	–
28	Chloroform : ethyl acetate (1 : 2)	–	–	–	–	–	–
29	Benzene : methanol (3 : 1)	–	–	–	–	–	–
30	Propanol : ethyl acetate : water (5 : 1 : 3)	0.2195 0.3171 0.4390	–	–	–	–	–
31	Chlorofom : methanol (1 : 1)	0.1222	0.0795	–	0.0739	–	–
32	Propanol : acetic acid : water (3 : 3 : 2)	0.8555 0.9222	–	–	–	–	–
33	Acetonitrile	0.0112	–	0.0667	–	–	–
34	Acetonitrile : water (2 : 2)	0.1149	0.1149	0.1111	0.1000	–	0.8888
35	Isopropanol	0.2280	–	–	–	0.7865	0.7752
36	Ethanol : water (7 : 3)	–	–	0.4444	–	–	–
37	Chloroform : methanol (9 : 1)	–	–	–	–	–	–
38	Chloroform : methanol (95 : 5)	0.1222	0.0795	–	–	–	–

Table 3. Identification of the chromatographic zones in the chromatograms of *N. umidischolae* no. 18 isolate

Run no.	Eluent	Suspension $R_f \pm 0.02$	Water-alcohol extract $R_f \pm 0.02$			Hexane extract $R_f \pm 0.02$	Methanol extract $R_f \pm 0.02$
			20%:80%	50%:50%	80%:20%		
1	Acetone	0.1904	0.1000 0.2125	0.0889	0.0344	–	–
2	Benzene	–	–	–	–	–	–
3	Benzene : methanol (1 : 1)	0.0930	–	–	–	–	0.0697
4	Benzene : methanol : acetic acid (1 : 1 : 1)	0.0778 0.2222 0.3444	–	0.8667	–	–	–
5	Butanol	0.6250	–	–	0.6746	–	–
6	Butanol : acetic acid (1 : 1)	0.6556	–	0.7889 0.9222	–	0.9438	–
7	Butanol : acetic acid : water (1 : 1 : 1)	0.7222	–	0.8202	–	–	–
8	Hexane	–	–	–	–	–	–
9	Hexane : ethyl acetate (1 : 1)	–	–	–	–	–	–
10	Methanol	0.1097	–	0.0769	0.0555	–	–
11	Acetic acid	0.1195 0.1829 0.3049 0.8902	0.7528	–	–	–	–
12	Chloroform	–	–	–	–	–	–
13	Chloroform : acetic acid (9 : 1)	0.0795	–	0.0787	–	–	–
14	Ethyl acetate	–	–	–	–	–	–
15	Ethanol : hexane : ethyl acetate (1 : 1 : 1)	–	0.1176	–	–	–	–
16	Ethanol : acetic acid (1 : 1)	0.4137 0.1724	0.8807	0.9000	–	–	–
17	Ethyl acetate : methanol : water (1 : 1 : 1)	0.1176	0.0833	–	0.1279	–	–
18	Ethyl acetate : tetrachloroethane : water (1 : 1 : 3)	0.7558	0.7558	–	–	–	–
19	Ethyl acetate : methanol (2 : 1)	–	–	–	–	–	–
20	Water : sodium citrate : citric acid (2 : 1 : 5)	0.8556	–	–	–	–	–
21	Butanol : water : ethanol (4 : 2 : 1)	0.2954	–	0.2584	0.3048	–	0.6790
22	Butanol : methanol (1 : 1)	–	0.7683	0.5115	0.5063	–	–
23	Ethanol : water (4 : 1)	0.3158 0.6974	–	0.7622	–	–	–
24	Ethanol : water (2 : 8)	0.9111	–	–	0.9213	–	–
25	Ethanol : water (5 : 5)	0.9111	–	0.4444	–	–	0.8977
26	Ethanol : water (8 : 2)	0.2857 0.6571	–	0.8778	0.7073	–	0.7857
27	Methanol : benzene : chloroform (4 : 2 : 1)	–	–	–	–	–	–
28	Chloroform : ethyl acetate (1 : 2)	–	–	–	–	–	–
29	Benzene : methanol (3 : 1)	–	–	–	–	–	–
30	Propanol : ethyl acetate : water (5 : 1 : 3)	0.3255	–	0.8764	–	–	–
31	Chlorofom : methanol (1 : 1)	0.1222	–	0.0667	–	–	–
32	Propanol : acetic acid : water (3 : 3 : 2)	0.7222	–	0.7865	–	–	–
33	Acetonitrile	0.0795	0.0843	–	0.0224 0.1378	–	–
34	Acetonitrile : water (2 : 2)	0.0805	0.0714	0.1236	0.1279	–	0.8414
35	Isopropanol	0.3376	–	0.1282 0.9103	–	–	0.1093
36	Ethanol : water	–	–	0.4444	–	–	–
37	Chloroform : methanol (9 : 1)	–	–	–	–	–	–
38	Chloroform : methanol (95 : 5)	0.1222	–	1.0667	–	–	–

Table 4. Determination of the substance group based on R_f factors and eluents (according to Kirchner, 1978)

Run no.	Eluent	Substance group	Sample name	
			p. Nocardiopsis no. 2	*p. Nocardiopsis* no. 18
1	Acetone	Pyridine derivatives : γ-pyridinecarboxylic acid, α-pyridinecarboxylic acid	Water-alcohol extract (50% : 50%) R_f 0.0562	–
2	Ethanol : water (7 : 3)	Peptides : hydroxyproline	Water-alcohol extract (50% : 50%) R_f 0.4444	Water-alcohol extract (50% : 50%) R_f 0.4444
3	Chloroform : methanol (95 : 5)	Antibiotics : narbomycin	Suspension R_f 0.1222	
		Antibiotics : foromacidin C	–	Water-alcohol extract (50% : 50%) R_f 1.0667
		Antibiotics : tylosin	Water-alcohol extract (20% : 80%) R_f 0.0795	–
4	Methanol	Antibiotics : erythromycin	Suspension R_f 0.1666	–
5	Benzene : methanol (1 : 1)	Phenols : protocatechuic aldehyde	Water-alcohol extract (80% : 20%) R_f 0.0602	Methanol extract R_f 0.0697

For each chromatographic zone the R_f factor was calculated (Tables 2 and 3).

In the suspension and extracts of *N. umidischolae* no. 2 isolate, separation was achieved with 26 out of 38 eluents of different polarities. For the isolate suspension the best separation and quality of chromatographic zones were afforded by the benzene : methanol : acetic acid (1 : 1 : 1), acetic acid, propanol : ethyl acetate : water (5 : 1 : 3), and propanol : acetic acid : water (3 : 3 : 2) elution systems.

In the case of the water-alcohol extract, separation was effected by the following eluting solvents : acetone, benzene : methanol (1 : 1), butanol, ethyl acetate : methanol : water (1 : 1 : 1), butanol : water : ethanol (4 : 2 : 1), butanol : methanol (1 : 1), ethanol : water (2 : 8), chloroform : methanol (1 : 1), acetonitrile, acetonitrile : water (2 : 2), ethanol : water (7 : 3), and chloroform : methanol (95 : 5).

For the hexane extract separation was afforded by isopropanol at $R_f = 0.7865$.

In the methanol extract, separation was achieved with the following eluting solvents : benzene : methanol (1 : 1), butanol : acetic acid (1 : 1), butanol : water : ethanol (4 : 2 : 1), ethanol : water (5 : 5), acetonitrile : water (2 : 2), and isopropanol.

Eluting solvents most suitable for the separation were acetone, butanol : water : ethanol (4 : 2 : 1), butanol : methanol (1 : 1), and acetonitrile : water (2 : 2) which effected separation in four to five out of six samples. The butanol : water : ethanol (4 : 2 : 1) system afforded separation in the suspension at $R_f = 0.2816$, in the 20% : 80% water-alcohol extract at $R_f = 0.2561$, in the 50% : 50% water-alcohol extract at $R_f = 0.3256$, in the 80% : 20% water-alcohol extract at $R_f = 0.2439$, and in the methanol extract at $R_f = 0.2250$.

Similar data were obtained from identification of zones in the chromatograms recorded for *N. umidischolae* no. 18 isolate (Table 3).

Separation in the suspension and extracts from isolate of *N. umidischolae* no. 18 strain was achieved with 28 out of 38 eluents of different polarities.

The best separation in the case of the suspension of *N. umidischolae* no. 18 isolate was effected by the following elution systems : benzene : methanol : acetic acid (1 : 1 : 1); acetic acid; ethanol : acetic acid (1 : 1); ethanol : water (4 : 1); and ethanol : water (8 : 2).

For the three water-alcohol extracts from the isolates the most suitable eluent was acetone ($R_f = 0.1000$, $R_f = 0.2125$, $R_f = 0.0889$, $R_f = 0.0344$), butanol : methanol (1 : 1) ($R_f = 0.7683$, $R_f = 0.5115$, $R_f = 0.5063$), and acetonitrile : water (2 : 2) ($R_f = 0.0714$, $R_f = 0.1236$, $R_f = 0.1279$).

In the case of the hexane extract separation was afforded by the butanol : acetic acid (1 : 1) system at $R_f = 0.9438$.

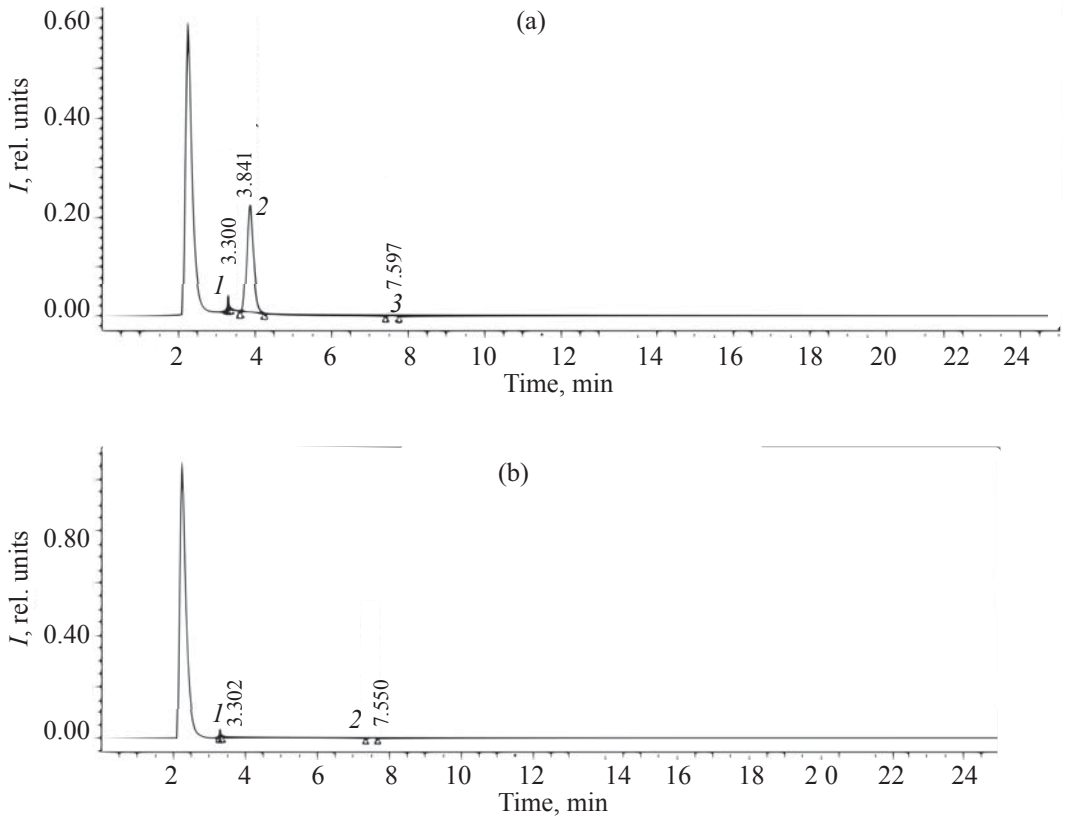

Fig. 1. HPLC chromatograms of the water-alcohol extracts (20%:80%) from (a) *N. umidischolae* no. 2: (*1*) isocitrate, (*2*) acetic acid, (*3*) fumaric acid and (b) *N. umidischolae* no. 18: (*1*) isocitrate and (*2*) fumaric acid.

Separation in the methanol extract was achieved using the following eluting solvents : (a) benzene : methanol (1 : 1), butanol : water : ethanol (4 : 2 : 1), (b) ethanol : water (5 : 5), (c) ethanol : water (8 : 2), (d) acetonitrile : water (2 : 2), and (e) isopropanol.

The TLC technique is suitable for analysis of both volatile and nonvolatile compounds and is applicable to vitamins, steroids, drugs, synthetic organic materials, paints, essential oils, resins, and pesticides. In many cases this method provides the key to solving intricate practical problems [25]. Knowledge of R_f and of the eluent enables identification of the suspected components revealed by TLC analysis (Table 4).

In the case of the water-alcohol extract (50% : 50%) of the isolate no. 2, pyridine derivatives such as γ-pyridine carboxylic acid and α-pyridine carboxylic acid (R_f = 0.0562) were identified using acetone as eluting solvent [25]. With ethanol : water (7 : 3) elution system, hydroxyproline from peptide group (R_f = 0.4444) was identified. Using the chloroform : methanol (95 : 5) system, antibiotic narbomycin (R_f = 0.1222) was detected in the suspension of the isolates, antibiotic tylosin, in the water-alcohol extract (20% : 80%) from *p. Nocardiopsis* no. 2 isolate (R_f = 0.0795), and antibiotic foromacidin C (R_f = 1.066), in the water-alcohol extract (50% : 50%) from *p. Nocardiopsis* no. 18. Antibiotic erythromycin was identified in the *p. Nocardiopsis* no. 2 isolate suspension using methanol (R_f = 0.1666). With benzene:methanol (1 : 1) eluting solvent, protocatechuic aldehyde from phenol group was detected in the water-alcohol and methanol extracts.

These substance groups have much significance for plant growth applications, specifically for plant protection against insect pests and phytopathogens of various etiologies.

Study of the component structure of the metabolites of *N. umidischolae* strain no. 2 by HPLC revealed the presence of the following organic acids: isocitric, acetic, fumaric, malic, lactic, and citric acids (Table 5, Figs. 1a, 2a, and 3a). In the case of *N. umidischolae* no. 18 strain the organic acids revealed were isocitric, acetic, fumaric, and lactic acids (Table 5, Figs. 1b, 2b, and 3b).

Table 5. Organic acids in the water-alcohol extracts from *N. umidischolae* no. 2

Trivial name	IUPAC name	Content, g/L		
		20% : 80%	80% : 20%	50% : 50%
Isocitric acid	1-Hydroxy-1,2,3-propanetricarboxylic acid	0.460	–	–
Acetic acid	Ethanolic acid	51.448	14.392	0.337
Fumaric acid	*trans*-Butenedioic acid	0.001	0.002	0.001
Maleic acid	2-Hydroxybunanedioic acid	–	0.029	–
Lactic acid	2-Hydroxypropanoic acid	–	0.168	–
Citric acid	2-Hydroxy-1,2,3-propanetricarboxylic acid	–	0.003	–

The HPLC analysis showed that, in the water-alcohol extracts of *N. umidischolae* strain no. 2, there were six organic acids of which acetic acid was detected in the largest amount of 0.337–51.448 g/L in the three variants of the water-alcohol extracts. Acetic acid is an organic compound, a weak saturated monobasic carboxylic acid which is used as a reaction medium for oxidation of various organic substances.

Fumaric acid was also detected in the three variants of the water-alcohol extracts in amounts ranging from 0.001 to 0.002 g/L. Isocitric acid was identified in the 20% : 80% water-alcohol extract in the amount of 0.460 g/L. HPLC analysis of the 80% : 20% water-alcohol extract showed the presence of malic (0.029 g/L), lactic (0.168 g/L), and citric (0.003 g/L) acids. Fumaric acid, *trans* isomer of butenedioic acid, exhibits bactericidal and antiseptic properties.

Four organic acids were identified by the HPLC chromatograms recorded for *N. umidischolae* no. 18 isolate (Table 6).

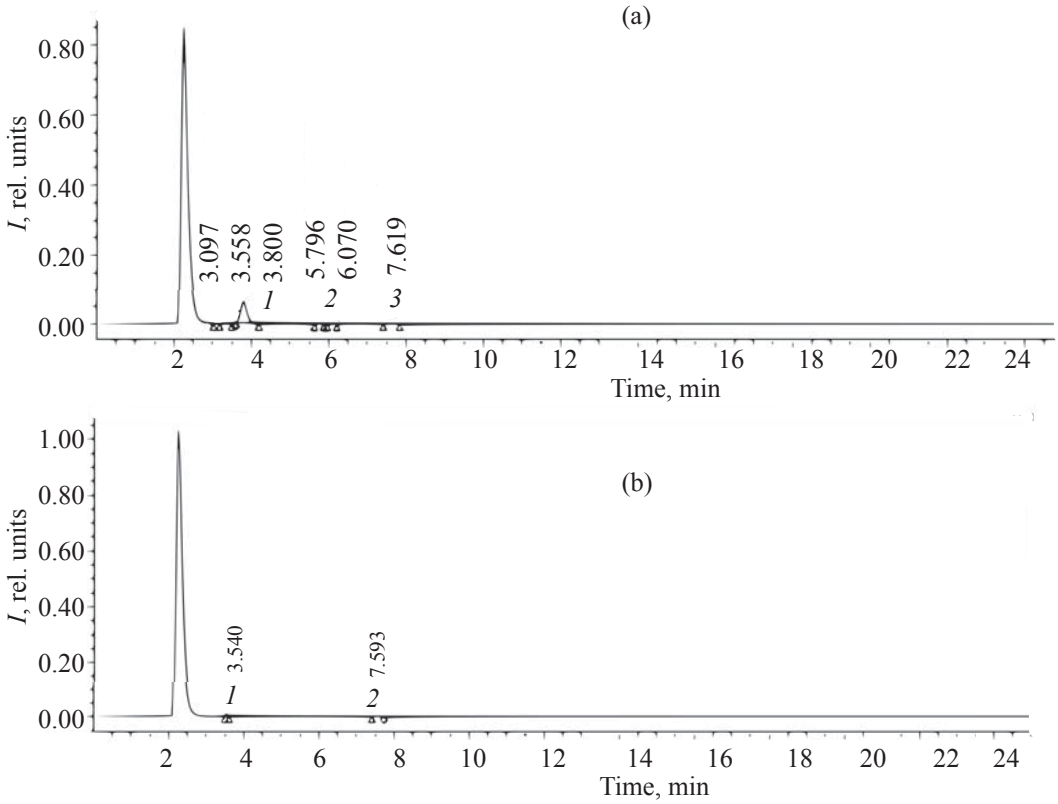

Fig. 2. HPLC chromatograms of the water-alcohol extracts (80% : 20%) from (a) *N. umidischolae* no. 2: (*1*) acetic acid, (*2*) citric acid, and (*3*) fumaric acid and (b) *N. umidischolae* no. 18: (*1*) lactic acid and (*2*) fumaric acid.

Table 6. Organic acids in the aqueous-alcoholic extracts from *N. umidischolae* no. 18

Trivial name	IUPAC name	Content, g/L		
		20% : 80%	80% : 20%	50% : 50%
Isocitric acid	1-Hydroxy-1,2,3-propanetricarboxylic acid	0.449	–	–
Acetic acid	Ethanolic acid	–	–	0.337
Fumaric acid	*trans*-Butenedioic acid	0.001	0.001	0.001
Lactic acid	2-Hydroxypropanoic acid	–	0.184	–

The highest content of isocitric acid, 0.449 g/L, was detected for the 20% : 80% water-alcohol extract. The lowest content of fumaric acid (0.001 g/L) was recorded from all the three variants of the water-alcohol extract.

Acetic acid was found in the 50% : 50% water-alcohol extract in the amount of 0.337 g/L, and lactic acid, in the 80% : 20% water-alcohol extract (0.184 g/L).

The HPLC data for the 20% : 80% water-alcohol extracts are indicative of the presence of the same organic acids, isocitric and fumaric, in the two isolates (Fig. 1). However, along with these acids this water-alcohol extract from *N. umidischolae* no. 2 strain contained a sufficiently large amount of acetic acid (51.448 g/L). Isocitric acid is a tricarboxylic acid, a structural isomer of citric acid, having high antioxidant activity.

Analysis of the 80% : 20% water-alcohol extracts from the two strains revealed the same organic acids, fumaric and lactic (Fig. 2). Lactic acid (lactate) belongs to the family of carboxylic acids; this is an alpha-hydroxy acid possessing antibacterial properties. HPLC analysis of the water-alcohol extract from *N. umidischolae* strain no. 2 revealed, along with the above-mentioned acids, three other acids : acetic (14.392 g/L), malic (0.029 g/L), and citric (0.003 g/L). Citric acid is a tribasic carboxylic acid, a synthetic or natural antioxidant by its action. Malic acid is a dibasic oxycarboxylic acid from the class of fruit acids; it exhibits powerful antioxidant and bactericidal properties.

As seen from the HPLC chromatograms of the 50% : 50% water-alcohol extracts (Fig. 3), analyses of the two

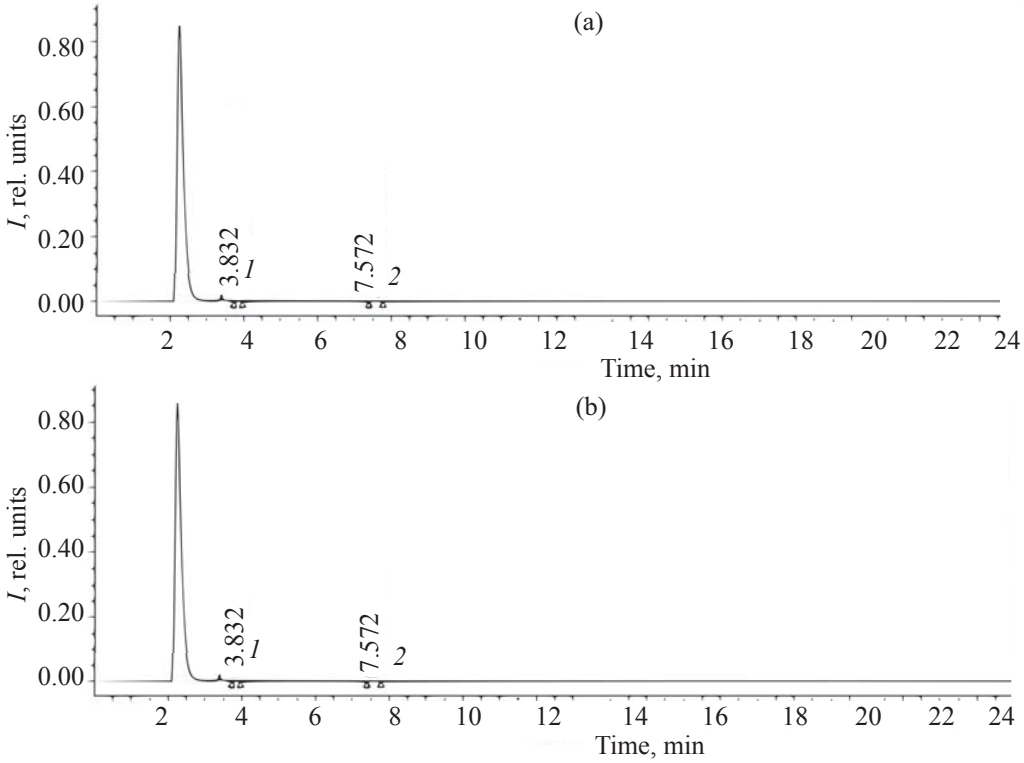

Fig. 3. HPLC chromatograms of the water-alcohol extracts (50% : 50%) from (a) *N. umidischolae* no. 2: (*1*) acetic acid and (*2*) fumaric acid and (b) *N. umidischolae* no. 18: (*1*) acetic acid and (*2*) fumaric acid.

strains gave identical results. Specifically, these extracts contained acetic (0.337 g/L) and fumaric (0.001 g/L) acids.

CONCLUSIONS

Thin layer chromatographic analysis of the metabolites of the *N. umidischolae* nos. 2 and 18 strains using 38 eluting solvents and qualitative tests was carried out. The analysis confirmed the presence of a broad range of substances with different R_f factors both in the suspension and in the water-ethanol (in three variants : 20% : 80%, 50% : 50%, 80% : 20%), methanol, and hexane extracts.

Glycosides, alkaloids, and flavonoids were detected in the metabolites of *N. umidischolae* nos. 2 and 18 isolates.

TLC analysis of the suspension and the extracts of the culture liquid of the isolates revealed the presence of the following substance groups : pyridine derivatives γ-pyridinecarboxylic acid and α-pyridinecarboxylic acid; protocatechuic aldehyde, having antioxidant properties, from phenol group; antibiotics tylosin, possessing antibacterial properties, erythromycin, foromacidin C, and narbomycin, having bactericidal and bacteriostatic activity; and hydroxyproline from peptide groupThese substance groups have much significance for plant growth applications, specifically as plant protection agents effective against insect pests and phytopathogens of various etiologies.

Study of the component structure of the metabolites of bacteria *N. umidischolae* no. 2 by high-performance liquid chromatography showed the presence of isocitric, acetic, fumaric, malic, lactic, and citric acids. Isocitiric, acetic, fumaric, and lactic acids were detected in the metabolites of *N. umidischolae* no. 18 culture. The components of the metabolites of the studied strains, identified by high performance liquid chromatography, are organic acids that are extensively applied in biotechnological production.

Thus, isolate nos. 2 and 18 represented by the *N. umidischolae* species were analyzed by means of qualitative tests and thin layer and high-performance liquid chromatography techniques. It was found that these isolates are a rich source of biologically active substances with diverse chemical structures and action spectra. They are promising candidates for agrobiotechnological applications, specifically as a basis of biological control agents effective against a wide range of insect pests.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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