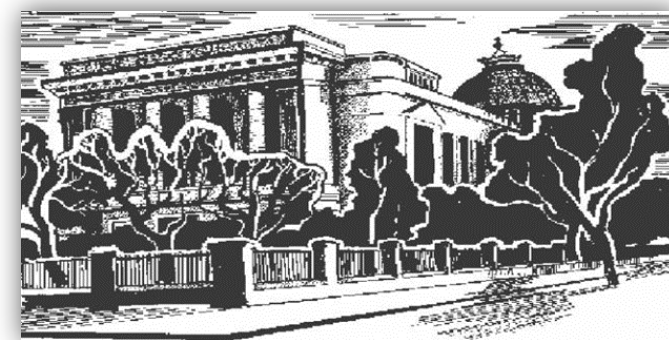


Isolation, Purification and Antifungal Activity of Daidzeins Produced by Halotolerant *Streptomyces sampsonii* INA01478



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The genus *Streptomyces* belongs to the phylum Actinomycetota. The representatives are Gram positive mycelial and spore-forming bacterium with high GC content. The *Streptomyces* genus is produced chemical molecules with high structural diversity a range of biological activities, such as antimicrobial, antifungal, antitumor, anti-inflammatory, and insecticidal properties. Recently, there has been significant increase in the investigation of microorganisms that inhabit extreme ecological niches such as mountainous regions, caves, deserts, marine sediments, and frozen soils.

The halotolerant *Streptomyces sampsonii* INA 01478 strain derived from the bottom sediments of Trondheim Fjord, the Norwegian Sea, was investigated. The *Streptomyces sampsonii* strain INA 01478 was grown on oatmeal agar, Gause 1 and Gause 2 media. The strain had the best growth and formed the obvious aerial mycelium on oatmeal agar at a salt concentration from 0 to 5% NaCl. The formation of aerial mycelium decreased at 7% NaCl concentration. The melanoid pigments did not formed (Fig. 1).

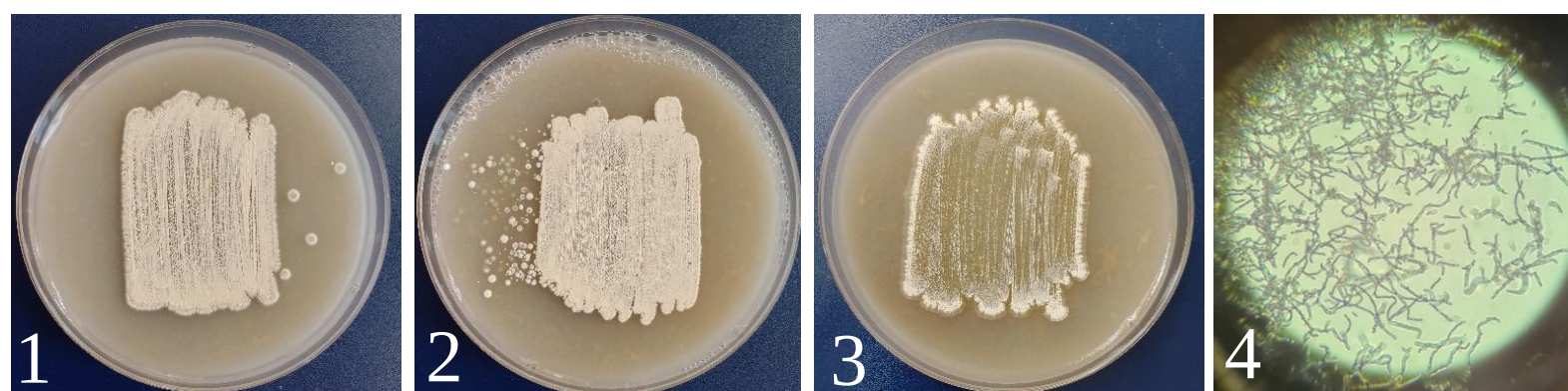
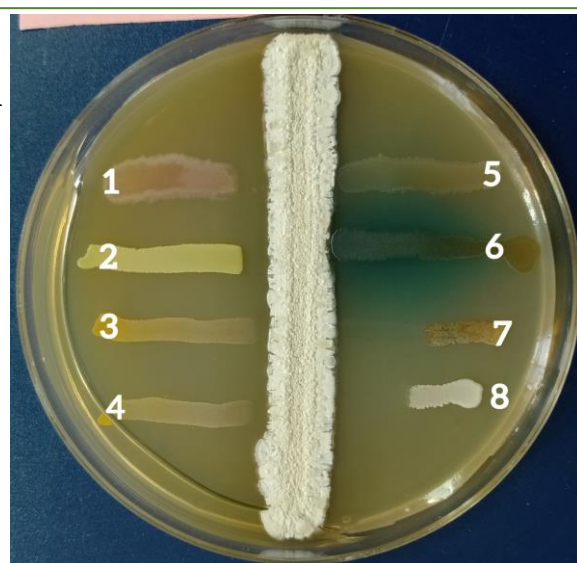


Figure 1. The growth of *Streptomyces sampsonii* INA 01478 on oatmeal agar at a salt concentration: 1 –3% NaCl, 2 –5% NaCl, 3 - 7% NaCl; 4 – microscopic morphologies of aerial mycelium.

It was found that the metabolites produced by the had pronounced antifungal activity against the *Candida albicans* ATCC 14053, *Saccharomyces cerevisiae* ИНА 01042, *Fusarium oxysporum* VKPM F-148, *Aspergillus niger* ATCC 16404. The spectrum of antibacterial activity was studied using test cultures of gram-positive bacteria strains - *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 43300, *Micrococcus luteus* ATCC 9341 and gram-negative bacteria - *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 (Fig. 2).

Figure 2. Antimicrobial activity of *Streptomyces sampsonii* INA 01478 on Gause 2 medium against test cultures:

1. *B. subtilis* ATCC 6633 ,
2. *M. luteus* ATCC 9341,
3. *S. aureus* ATCC 6538,
4. *S. aureus* ATCC 43300,
5. *E. coli* ATCC 25922,
6. *F.aeruginosa* ATCC 27853,
7. *Sac. cerevisiae* INA 01042,
8. *C. albicans* ATCC 14053



To obtain bioactive compounds, the strain was grown on a liquid medium A4 (soy flour – 10.0 g/l, glucose – 10.0 g/l, NaCl – 5.0 g/l, CaCO₃ – 2.5 g/l). Culture liquids were separated from biomass by centrifugation at 4800 rpm for 20 min. The culture supernatant was extracted by petroleum ether, ethyl acetate, butanol (Fig. 3).

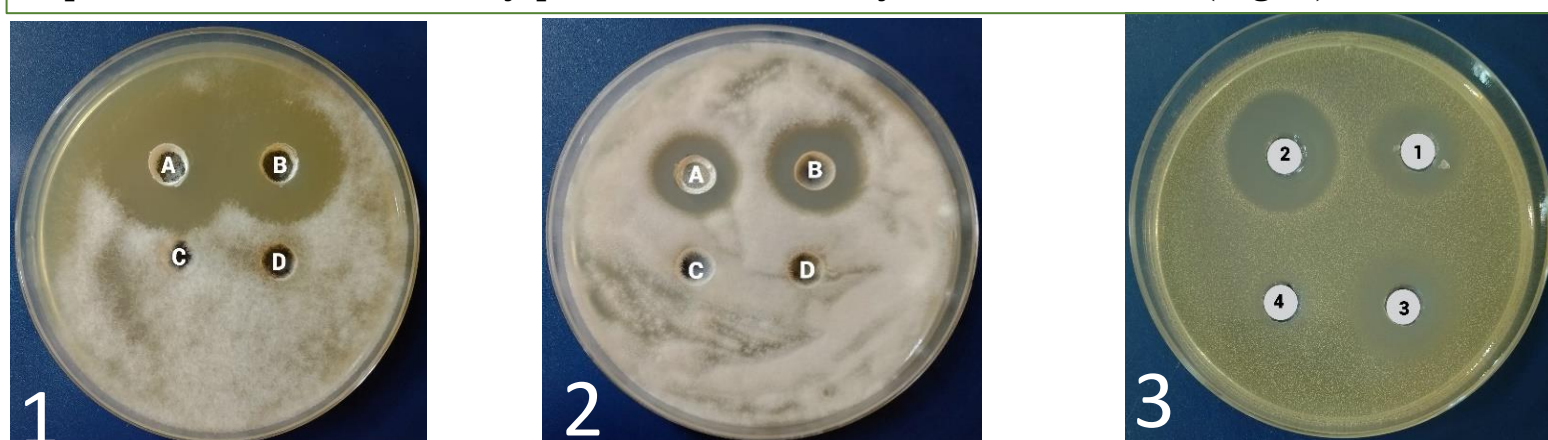


Figure 3. Antifungal activity *Streptomyces sampsonii* INA 01478 against: 1. *F. oxysporum* VKPM F-148 (A,B - culture supernatant, C,D – control medium); 2. *Aspergillus niger* ATCC 16404 (A,B - culture supernatant, C,D – control medium); 3. *C. albicans* ATCC 14053: 1 - extract by petroleum ether, 2 - extract by ethyl acetate, 3 - extract by butanol, 4 – control after extract.

Analysis of biologically active metabolites using HPLC, NMR, and MS data analysis revealed that the component of the subfraction is the isoflavonoid daidzein ([7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one]) (Tab. 1, Fig. 4, Fig. 5).

Table 1– Antimicrobial activity of different fraction from crude ethyl acetate extract of *Streptomyces sampsonii* INA 01478

№ fraction	Zone of growth inhibition, mm				
	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>C. albicans</i> ATCC 2091	<i>F. oxysporum</i> VKPM F-148	<i>A. niger</i> ATCC 16404
1	n/a	n/a	n/a	n/a	n/a
2	n/a	n/a	n/a	n/a	n/a
3	n/a	n/a	15	17	11
4	n/a	n/a	17	20	12
5	n/a	n/a	12	15	n/a
6	n/a	n/a	12	11	n/a
7	n/a	n/a	14	n/a	n/a

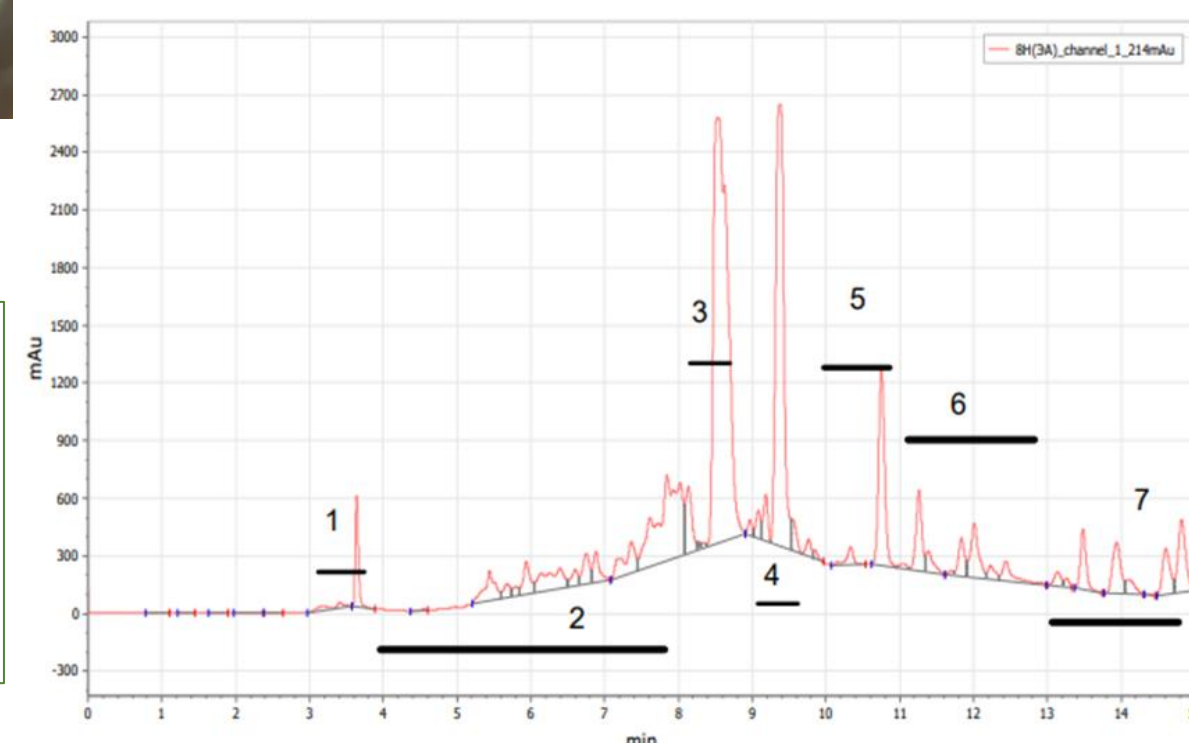


Figure 4. Fractionation of the *Streptomyces sampsonii* INA 01478 concentrate by analytical reversed-phase HPLC. The target component is marked by number 3.

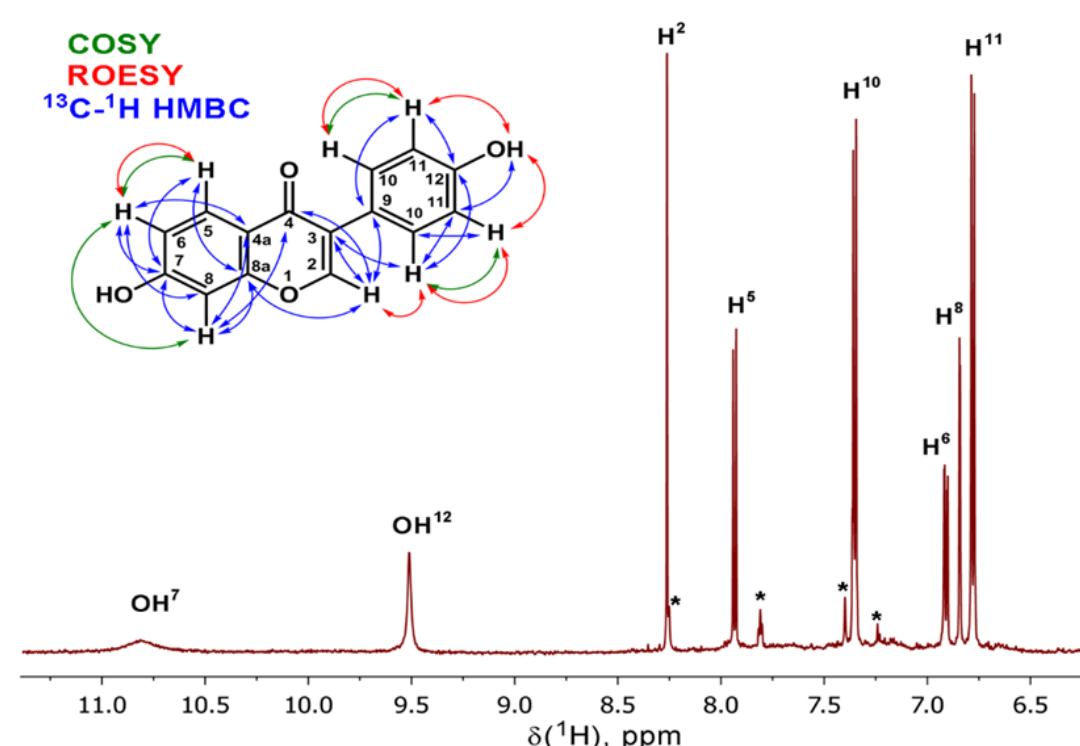


Figure 5. Fragment of the 1H NMR spectrum of daidzein recorded in DMSO-d₆ at 600 MHz and 298 K, with signal assignments. Asterisks indicate impurity signals. Arrows represent ¹H-¹H and ¹³C-¹H correlations observed in 2D NMR spectra: DQF-COSY (green arrows), ROESY (red arrows), and ¹³C-¹H HMBC (blue arrows).