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Antimicrobial activity of *Polyscias filicifolia* cell biomass extracts

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Antibacterial activity of extracts of *Polyscias filicifolia* biomass from bioreactor and callus was determined using the agar disc-diffusion method. The microorganisms *Staphylococcus aureus* (three strains) showed the highest sensitivity to extracts of *P. filicifolia* biomass from a bioreactor. The values were comparable with nitrofurantoine used as a standard. *Micrococcus flavus, Sreptococcus pyogenes* and *S. agalatiae* were less sensitive. The effect of *P. filicifolia* callus extract on the above bacteria was less pronounced than that of extracts of biomass from a bioreactor.

1. Introduction

Polyscias filicifolia Bailey *(Araliaceae)* grows in the tropical and subtropical climate. The genus *Polyscias* includes ca. 100 species, some of which are used by inhabitants of those regions as aromatics and food supplements. Four species : *P. balfouriane, P. dichrosta, P. filicifolia and P. fruticosa* are used medicinally [1]. *P. fruticosa* is used in Vietnam as a tonic and to increase the resistance to various infections. Extracts of *P. fruticosa* are known to stimulate lactation and promote healing of wounds and are also used as a remedy for neuralgia and arthritis. The most interesting species is *P. filicifolia,* which is included in the National Vietnamense Pharmacopoeia as a tonic and a cardiac tonic [1]. Trilis and Davidov [2] described the adaptogenic activity of *P. filicifolia* extracts, helping the body adapt to stress.

The main active constituents of the *Polyscias* species are triterpenoid glycosides [3]. Most of them contain oleanolic acid or hederogenin as aglicons. A few years ago polyacetylene compounds were isolated from rhizomes of *P. fruticosa*. These were panaxydol, farcarinol and heptadeca-1,8-(*E*)-diene-4,6-diyne-3,10-diol [4, 5].

Some specific pharmacological activities of these compounds were described by Ahn and Kim [5] who noticed cytotoxic action of heptadeca-1,8(*E*)-diene-4,6-diyne-3,10-diol and Lutomski et al. [4, 5] who were the first to demonstrate that the compounds and farcarinol possess antimicrobial and antifungal properties. Lutomski et al. [5] calculated the MIC values of the compounds. They observed a very strong action against gram-positive cocci: *Streptococcus pyogenes* and *Staphylococcus aureus, S. epidermidis, S. faecalis,* and dermatophytes (*Microsporum gypseum* K_1).

The demand for antimicrobial, antistress and immunomodulatory drugs led to the initiation of tissue cultures of *Polyscias* species.

The first results in the area of *Polyscias* tissue cultures were obtained by Slepian et al. [7, 8], the work was continued by Michailova and Slepian [9] and Kotin [10]. In the period 1995–2000 suspension cultures of *Polyscias filicifolia* were obtained by Klushin et al. [1], who also improved the method of cell culture in a bioreactor.

In 1996 the Russian dietary supplement "Vitagmal" containing *P. filicifolia* extract (produced by SMC "Biopharmtox", S. Petersburg) was introduced onto the market as a tonic, antistress and immunomodulator.

The antimicrobial activity in vivo was investigated by Docenko and Kotin [11] in the Department of Pharmacological Prophylaxis, Therapy and Innovations, "Ear, Throat, Nose and Speech Institute" in S. Petersburg, Russia.

P. filicifolia extracts were tested in 16 patients (18–45 years old). The dose was: 10–12 drops of the extract sublingually 1 h before breakfast for 14 days. Subsequently, bacterial species were identified and the number of colonies was determined. Authors observed the effect of the extracts on the microflora of the palatine tonsils in the study patients. The counts of the pathogens *Staphylococcus aureus* and *Streptococcus pyogenes* decreased and normal microflora became dominant.

Oleanolic acid was found in *P. filicifolia* suspension culture from a bioreactor and callus growing on solid medium. TLC and gas chromatography were used for phytochemical analysis [12].

The aim of our study was to investigate the effect of plant cell culture extracts of *P. filicifolia* (cultured in a bioreactor), 1-year-old callus induced from *P. filicifolia* leaves and oleanolic acid on different microorganisms.

2. Investigations, results and discussion

Extracts of *P. filicifolia* biomass from a bioreactor, callus and oleanolic acid showed different effects on infective organisms. The results are presented in Table 1. Of 15 bacterial strains selected for the experiment ten gram positive strains were sensitive to the extracts of *P. filicifolia* tested: *Staphyloccus aureus* NCTC 4163, *S. aureus* ATCC 6538P, *S. aureus* ATCC 25932, *Bacillus sthearothermophilus* ATCC 7953, *B. cereus* ML 98, *B. subtilis* ATCC 6633, *Streptococcus pyogenes* (clinical group A), *S. agalatiae* (clinical), *Enterococcus faecium* ATCC 6057, and *Micrococcus flavus* NCIB 8166.

The diameter of the inhibited growth area ranged from traces to 30 mm. The highest activity was observed for *S. aureus* ATCC 25932 and *S. aureus* NCTC 4163. It is very important to notice that the high antibacterial activity of suspension culture extracts from a bioreactor was comparable to that of nitrofurantoine used as a standard.

Only trace growth inhibition was seen for *Bacillus cereus* ML 98 and *B. subtilis* ATCC 6633. Lower activity was noticed for callus initiated from leaves of *P. filicifolia* plant (0.5 m high growing in the Warsaw Botanical Garden). The growth inhibition area was only 12–16 mm (Table 1).

Oleanolic acid found in both plant tissues was not active in that test.

Table 1: Sensitivity of different gram positive bacteria to the extracts of *Polyscias filicifolia* biomass

Plant material (dry extract) of chemical compound	*Staphylococcus aureus*			*Bacillus*			*Streptococcus*		*Entero-coccus faecium*	*Micro-coccus flavus*
	NCTC 4163	ATCC 6538P	ATCC 25932	*cereus* ML 98	*stearother-mophilus* ATCC 7953	*subtilis* ATCC 6633	*pyogenes* (clinical)	*agalatiae* (clinical)	ATCC 6057	NCIB 8166
Cell biomass from bioreactor	28*/1.6	20/3.3	30/1.0	traces	12/>4.0	traces	18/3.6	18/3.6	16/>4.0	20/3.0
Callus (initiated from leaf) cultivated on NN medium	13/>4.0	13/>4.0	12/>4.0	0	0	0	16/3.6	14/>4.0	12/>4.0	16/3.6
Nitrofurantoine (0.3 mg/disc)**	20/0.120	20/0.120	23/0.120	21/0.120	27/0.100	24/0.120	26/0.100	22/0.120	20/0.120	26/0.1
Oleanolic acid	0	0	0	0	0	0	0	0	0	0

* – diameter (mm) of growth inhibition area, values shown are results of three individual experiments/MIC value expressed in mg of extract/ml
0 – inhibition of bacteria growth not observed,
** – used as a standard

Table 2: Determination of genotoxicity of *Polyscias filicifolia* tissues extracts and of oleanolic acid using the "rec-assay *Bacillus subtilis* test" according to Kada et al. [20]

Plant material	Bacillus subtilis	
	M45 rec$^-$ (mm)	H17 rec$^+$ (mm)
Biomass of *P. filicifolia* from bioreactor	0	0
Callus growing on solid NN medium in Erlenmeyer flask	0	0
Oleanolic acid	0	0
4-Nitroquinoline *N*-oxide	22	12

The gram negative bacteria *Pseudomonas aeruginosa* NTCC 6149, *Escherichia coli* NCTC 8196, *Klebsiella pneumoniae*, as well as the fungus *Candida albicans* ATCC 10231 were not sensitive in three tested samples.
Our observations confirmed clinical studies by Docenko and Kotin [11] in which the counts of *Staphyloccocus aureus* and *Streptococcus pyogenes* pathogen strains were decreased after treatment with extracts of *P. filicifolia* tissues. Our results are also in accordance with those obtained by Lutomski et al. [5] who showed the activity of two chemical compounds isolated from *Polyscias fruticosa* against some microorganisms.
Interestingly, all studied samples were not genotoxic (Table 2), which is very important for further biological and phytochemical studies.

3. Experimental

3.1. Plant cell culture from a bioreactor

In this study the plant cell culture of *P. filicifolia* strains BPNT-01-95 was used. This strain is included in the Russian Collection of Higher Plant Cell Culture (RCHPC) which is a member of the World Federation of Culture Collection (Nr 58).
Cell culture was maintained in the microbial bioreactors vol. 5 l (MF-107, New Brunswick with turbine type impeller) and 50 l (Electrolux El-75 with marine-type). Agitation speed in different stages of growth was 50–150 rpm.
Aeration rate was 0.1–0.5 VVM depending on the stage of growth. The temperature of cultivation was 26 °C, and pH and PO_2 were controlled. Murashige and Skoog [13] (MS) medium with kinetin and NAA, thiamin, mesoinositol and 3% sucrose was used. During the growth cycle dry and fresh biomass was measured (Fig.).
Specific growth rate in the exponential phase, growth index I (gram of dry weight/l), doubling time T (number of cells/24 h), and productivity P(g of d.w./l/24 h) were also calculated [1].

3.2. Callus culture

For comparison the callus from eight passage cultivated on Nitsch and Nitsch [14] (NN) medium with BAP, IBA, thiamine, mesoinositol and 2% of sucrose was used [12]. The growth rate of callus after 4 weeks was 185%.

3.3. Oleanolic acid

Pure compound oleanolic acid was tested for antimicrobial activity. The compound was used by Bloch [12] for phytochemical analysis of *P. filicifolia* leaves and tissue culture.

3.4. Microorganisms

The antimicrobial activity of ethanolic extracts obtained from the investigated samples were tested by the agar disc-diffusion method using Mueller-Hinton agar medium under standard conditions as described by NCCLS [15]. The following microorganisms were used: gram positive *Staphylococcus aureus* NCTC 4163, *S. aureus* ATCC 6538P, *S. aureus* ATCC 25932, *Bacillus cereus* ML 98, *B. sthearothermophilus* ATCC 7953, *B.subtilis* ATCC 6633, *Streptococcus pyogenes* (clinical strain), *S. agalatiae* (clinical strain), *Enterococcus faecium* ATCC 6057, *Micrococcus flavus* NCIB 8166; gram negative *Escherichia coli* NCTC 8196, *Klebsiella pneumoniae* (ESBL –producing clinical strain), *Pseudomonas aeruginosa* NCTC 6749 and fungi *Candida albicans* ATCC 10231 and *Rhodotorula rubra.*

3.5. Antimicrobial activity test

Lyophylised callus and biomass from a bioreactor were extracted with 96° ethanol. From 1 g of plant tissue 0.15 g of dry extract was obtained. For clinical investigation 10 g of biomass of "Vitagmal" in 100 ml of 40% ethanol was used.

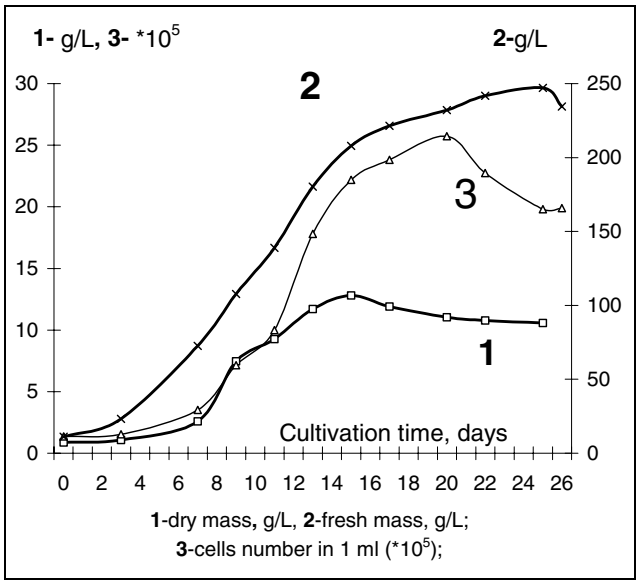

Fig.: Growth of *Polyscias filicifolia* cell culture in a 7.5 liter bioreactor

The Wathmann chromatography paper No 3 was used for sterile filter discs 9 mm in diameter for the diffusion method. Sterile filter paper discs were soaked in the test extract solutions using 0.5 mg of dry extract per one disc. The results were read after 18 h incubation at 35 °C. The sensitivity of different microorganisms to *Polyscias* extracts and oleanolic acid was expressed as the diameter of growth inhibition area. A control disc contained 0.3 mg/disc nitrofurantoine. Minimal inhibitory concentrations (MIC) were tested in Mueller-Hinton broth under standard conditions NCCLS [15]. This method is generally used for testing antimicrobial activity of different chemical compounds and plant extracts [16–18].

The microorganisms showing sensitivity in the disc-diffusion test were next used to determine genotoxic activity (Table 2). The genotoxicity test was performed according to Kada et al. [19, 20] rec-assay *Bacillus subtilis* test. In our experiments the test *Bacillus subtilis* strain M 45 rec^- possessing mutation in genes rec (of recombination of DNA repair) was more sensitive to genotoxic agents than strain H 17 rec^+ (the same but without mutation rec^-).

The three substances tested did not show any evidence of genotoxic activity, unlike the standard 4-Nitroquinoline N-oxide (Table 2).

3.6. Phytochemical analysis

Two methods described by Bloch [12]: TLC and GC were used for qualitative determination of oleanolic acid in cell biomass from a bioreactor and callus growing on solid medium.

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