Biological Activity of Water Extract from *Atractylodes macrocephala*

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The effects of water extract from *Atractylodes macrocephala* Koidz on biological activity were investigated. The crude water extract of *A. macrocephala* inhibited the growth of the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (3 mm inhibition zone at 300 μg/disc). However, it did not show growth inhibition activity against *Sreptococcus mutans* JC-2 (MIC >1,000 μg/mL). This extract was cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC_{50} 62.24 μg/mL at 150 μg/disc). These results suggest that water extract of *A. macrocephala* possesses antitumoral, and antimicrobial activities.

Key words : *Atractylodes macrocephala*, *Trichophyton mentagrophytes*, antimicrobial activity

Introduction

The rhizome of *Atractylodes macrocephala* Koidz (Compositae) is known as a tonic in China. It is reported as a nutrient for energy and stomach complaints and for treatment of dyspepsia and anorexia in the pharmacopeia of People's Republic of China. *Atractylodes macrocephala* (A. macrocephala) is used in Chinese folk medicine for the treatment of gastroenteric and splenic disorders[1]. Some *Atractylodes* plants, such as *A. japonica* and *A, lancea* are also used for pharmaceutical preparations in Japan, but two crude drugs are said to have different therapeutic effects, the former antisuderific activityand the latter diaphoretic activity[2]. The chemical constitutions of this plant investigated sesquiterpene and acetylene compounds[3]. Lin et al. described that *A. macrocephala* had a novel bissesquitpenoid, biatractylolide[4] .Choi et al[5]. reported that the crude extract of *A. macrocephala* was extracted with n-hexane, chloroform, ethyl acetate, methanol and water, succesively and the water extract had the strongest cytotoxicity against KB (ATCC No, OCL 17) and SK-MEL-3 (HBT 69) cell lines. The minimal inhibitory concentrations (MICs) of each solvent extract of *Atractylodes macrocephala* (*A. macrocephala*) against microoganisms were also examined. The antimicrobial activity of the ethyl acetate soluble extract of *A. macrocephala* had growth inhibition activity against *S. mutans* and *P. putida* (MICs, 500 μg/mL). Chun et al[6]. reported that the ethanol soluble extract of *A. macrocephala* inhibited tyrosinase activity and melanin contents with or without of α-MSH and forskolin *in vitro*. Melanin contents and tyrosinase activity have decreased in a dose-dependent manner. In this study, antitumor and antimicrobial activities of the crude water extract from *A. macrocephala* was examined.

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Materials and Methods

1. General experimental procedures

All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 50 ℃. Preparative silica gel TLC was carried out using Merck DC-plastikfolien Kieselgel 60 F_{254}, visualized with an UV lamp then by dipping in a vanillin solution (1 % vanillin, 1 % H_2SO_4 in EtOH) and heating. NMR spectrum of $CDCl_3$ solutions at 25 ℃ was recorded at 300 MHz for ^1H on a Varian VXR-300 spectrometer. Chemical shifts are given in ppm on the scale referenced to the solvent peaks of $CDCl_3$. ^1H-NMR referenced to 7.25 ppm.

2. Plant materials

The dried rhizomes of the herbal plant, *A. macrocephala* Koidz (Compositae), was obtained from a local herbal medicine store and taxonomically identified by Prof. Min Kyo Shin in the Department of Herbalogy, Wonkwang University. Voucher specimen of the plants are deposited at the Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University in Korea.

3. Preparation of the extract

A. macrocephala (11.55 g) was extracted with distilled water at reflux for three hours. The combined filtrates were evaporated under reduced pressure to give a dark brown gum (4.08 g, 35.32 %)which was stored at 4 ℃ until tested.

4. Screening for antiviral activity

The extract was applied (30 μL of a 10 mg/mL solution) to a small filter-paper disc, dried, and assayed for antiviral activity using Schroeder et al.s methods[7]. The results were observed either cell death (cytotoxicity), inhibition of virus replication, no effect (i.e., all of the cells show viral infection), or a combination of all three. The results were noted as the approximate size of the circular zone, radiating from the extract sample, from 1+ to 4+ representing 25% through to whole well sized zones. The notation used is inhibition/ antiviral activity. The type of antiviral effect, indicated by a number after the size of the zone, was also considered important and may give some indication as to the mode of cytotoxic action.

5. Screening for antibacterial and anti-yeast activities

Activity against the following bacterial strains and yeast was tested: multiresistant *Bacillus subtilis* (ATCC 19659), and *Candida albicans* (ATCC 14053). Extracts were dissolved and diluted in an appropriate solvent (usually ethanol : water) to a concentration of 10 mg/ml. Test plates are prepared from Mueller Hinton agar containing extract to give a final concentration of 100 μg extract/mL agar. Activity growing cultures of the test strains were diluted in saline so as to deliver 10^4 colony forming units onto the test, control (solvent), and blank (agar only) plates with a multipoint inoculator. Inoculated plates were incubated overnight at 37 ℃. Growth on the blank and control plates was checked and, if satisfactory, growth on the test plates was scored for each test strain as follows: (-) inhibition, no reduction in growth compared with the control, (+) inhibition, no growth.

6. Screening for antifungal activity

Activity against the following fungal strain was tested: *Trichophyton mentagraphytes* (ATCC 28185) local strain]. Fungal spore suspensions of the test organisms were applied to dextrose agar plates. Aliquots of the extract solutions were applied to filter paper discs, at 30 μg extract/disc, and dired at 37 ℃ for two hours. These discs were applied to the agar plates, two per plate, and incubated at 28 ℃.

7. Screening for cytotoxic activity

This is a measure of the ability of a sample to inhibit the multiplication of murine leukaemia cells. The sample was dissolved in a suitable solvent, usually ethanol, at 10 mg/mL, and 15 μL of this solution was placed in the first well of a multiwell plate. Seven two-fold dilutions were made across the plate. After addition of the cell solution, the concentration range in the test wells was 25,000 down to 195 ng/mL. After incubation for three days, the plates were read using an ELISA palte reader at 540 nm wavelength. Automated reading of the plates was possible with the addition of a MTT tetrazolium salt (yellow color). Healthy cells reduce this salt to MTT formazan (purple color)[8-9].

Results And Discussion

A crude water extract of *A. macrocephala* was prepared by grinding dried root of plant material and extracted with water. A crude extract was weak cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC_{50} 62.24 μg/mL @ 10 mg/mL at 150 μg/disc) and BSC monkey kidney cells (75 % activity @ 10 mg/mL at 300 μg/mL). Table 1 shows the antiviral activity against *Herpes simplex Type* I *virus* ATCC VR 733, (75 % activity @ 10 mg/mL, 300 μg/disc) and *Polio Type I virus* Pfizer vaccine strain, (50 % activity @ 10 mg/mL at 300 μg/disc). As indicated in Table 1, this crude extract which prepared from *A. macrocephala* inhibited the growth of the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (3 mm inhibition zone at 300 μg/disc). No activity was observed against the fungus *Candida albicans* ATCC 14053 and Gram-positive bacteria *Bacillus subtilis* ATCC 19659, (@ 10 mg/mL at 150 μ g/disc). *Streptococcus mutans* JC-2 is inactive with a MIC of >1,000 μg/mL. This water extract was found to have effective in vitro cytotoxic activity with IC_{50} of 62.24 μg/mL aganist P388 murine leukaemia cells ATCC CCL 46 P388D1. However, this extract was inactive against NIH 3T3 fibroblasts (IC50, 1,465 μg/mL). This water extract showed weaker antimicrobial activity than chloramphenicol and nystatin (Tables I and II)[4]. The crude water extract was analysed by ^{1}H-NMR spectroscopy of CDCl3 solubles. ^{1}H-NMR spectrum of the crude *A. macrocephala* contained the N-H and olefinic protons singals at δ 7.12 ppm brs and δ 5.61 - 5.36 ppm, two exomethylenic protons at δ 4.86 ppm d, and δ 4.71 ppm brs and two methyl proton signals at δ 0.94 ppm s and δ 1.85 ppm. This spectrum of the water extract showed weak signals at δ 3.65 and δ 4.10 ppm, which could be due to a methylene group unconjugated to a olefinic group and one methine proton signals at δ 1.56 ppm d. The ^{1}H-NMR spectrum of the water extract showed strong signals at δ 2.42 - 0.76 ppm, which could be due to methyl and methylene groups. The

1H-NMR spectrum of the water extract from *A. macrocephala* was indicative of the sesquiterpene lactam structure. As shown in Fig. 1, the crude extract from *A. macrocephala*-mediated cytotoxicity did not increase in the MTT method against NIH 3T3 fibroblasts when its crude extract was increased from 10 μg/mL to 250 μg/mL However, this water extract showed the cytotoxic activity in P388 murine leukaemia cells ATCC CCL 46 P388D1, and BSC monkey kidney cells[10].

Table 1. Biological activities of the crude extract from *A. macrocephala*

Extract	Cytotoxicity			
	BSCa	*Herpes simplex virus*[b]		*Polio virus*[b]
	+ + +	+ + +		+ +
	Antimicrobial activity[c]			
	B. subtilis	*C. albicans*	*T. mentagrophytes*	*S. mutans*[d]
Extract	-	-	HM 3	>1,000
Chloramphenicol	SM 14	0	0	0
Nystatin	0	SM 10	HM 6	0
	P388 cytotoxity			
Mitomycin C[e]	0.073			
Extract	62.238[f]			
	1.465[g]			

a% of well showing cytotoxic effects. @ 10 mg/mL, 300 μg/disc: + + +: 75 % activity. bCytotoxicity in antiviral assays. @ 10 mg/mL, 300 μg/disc: Zone of cytotoxic activity: + + +: 75 % activity; + +: 50 % activity. cWidth of zone of inhibition in mm: 300 μg/disc: -: no reduction in growth, 0: not determined. Chloramphenicol: 30 mcg/disc, Nystatin: 100 unit/disk. SM: Sharp margin, HM: Hazy margin, numbers refer to zone of inhibition (mm). dAntimicrobial activity by the broth dilution method using the Muller-Hinton broth (Oxoid/England)5) : MIC >1,000 μg/mL. eToxicity of sample to P388 murine leukaemia cells (ATCC CCL 46 P388D1) in μg/mL at 0.075 μg/disc. P388 : Concentration of the sample required to inhibit cell growth to 50 % of a solvent control. fToxicity of sample to P388 murine leukaemia cells (ATCC CCL 46 P388D1) in μg/mL at 150 μg/disc. gCytotoxicity of sample to NIH 3T3 fibroblasts in μg/mL.

Table 2. List of microorganisms used for antimicrobial susceptibility test

Gram-positive bacteria	
Bacillus subtilis	ATCC 19659
Streptococcus mutans	JC-2
Fungi	
Candida albicans	ATCC 14053
Trichophyton mentagrophytes	ATCC 28185

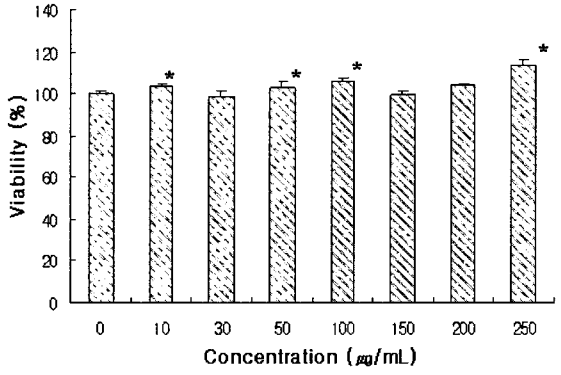

Fig. 1. In vitro cytotoxicity of the crude extract from A. macrocephala by the MTT method. This crude extract was serially diluted in RPMI-1640 with 10 % FBS and mixed with equal volume of NIH 3T3 fibroblasts (5 x 10^5). The colorimetric assay was performed as described in the materials and methods section. Data are mean of results obtained from four sets of experiments. Significantly different from the control values: *p<0.05 (Student's t-test).

In conclusion. the crude water extract of *A. macrocephala* inhibited the growth of the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (3 mm inhibition zone at 300 μg/disc). However, *Streptococcus mutans* JC-2 is inactive with a MIC of >1,000 μg/mL. This extract was cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC_{50} 62.24 μg/mL at 150 μg/disc). We suppose that this crude water extract of *A. macrocephala* is the antifungal activity. Further research is needed to the separation of the main bioactive components from the extract of plant and the results will be discussed elsewhere.

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