Natural Product Sciences
14(1) : 32-36 (2008)

Antimicrobial activity of Mongolian medicinal plants

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Abstract – The antimicrobial activity of seventy five ethanol extracts obtained from 67 different kinds of plant species of the Mongolian flora were evaluated by means of the disc diffusion method against five species of microorganisms, *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Micrococcus luteus* and *Pseudomonas aeruginosa.* Among the plant extracts examined, 34 kinds of extracts demonstrated significant antibacterial activity against one or more species of microorganisms, respectively. Especially, the root extract of *Paeonia anomala,* the whole herb extract of *Myricaria alopecuroides,* the whole herb extract of *Comarum zalesovianum,* the whole herb extract of *Agrimonia pilosa* and some other plant extracts demonstrated a particularly potent antimicrobial activity. The ethylacetate fractions obtained from the whole herb extract of *Myricaria alopecuroides* and from those of *Sedum aizoon, Paeonia anomala, Sedum hybridum* and *Dasiphora fruticosa* exhibited a particularly potent antibacterial activity especially against *Staphylococcus aureus* and *Micrococcus luteus.*

Keywords – Mongolian Medicinal plants, plant extract, antimicrobial activity, disc diffusion method, minimal inhibition concentration

Introduction

Mongolia has a very diverse and distinctive flora, which represent a mixture of species from the northern taiga of Siberia, the steppe, and the deserts of Central Asia. Many of them are unique to Mongolia and largely unknown to the rest of the world. There are registered 3,000 species of flowering plants. 975 species are registered as medicinal plants which are used in folk and traditional medicine of Mongolia and boundary countries, including 200 species which are used in modern medicine (Galbaatar, *et al.*, 1999, Ligaa *et al.*, 2006 and Sanchir *et al.*, 2003). The traditional and herbal medicines are known as essential resources of phytomedicines and nutraceuticals which could provide a variety of beneficial effects on human health in Mongolia. In fact, according to WHO, herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas or developing countries (WHO, 2001).

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Thus, over the last few decades, a lot of researches have been conducted which were focused mainly on the evaluation of new and alternative medicinal use of Mongolian medicinal plants for the purpose of development of new type of phytomedicines and nutraceuticals originated from Mongolian endemic plants. However, it costs vast of research fund, scientific equipments and facilities to examine various pharmaceutical or therapeutical property, even for evaluation of simple antimicrobial activity of Mongolian endemic plants. Thus, we employed a feasible and traditional disc diffusion method to quantify antimicrobial activity of plant extracts, due to its high sensitivity, simplicity, and cost-effectiveness.

In this paper, we described briefly the antimicrobial activities of 75 ethanol extracts obtained from 67 different Mongolian medicinal plants against bacteria species *Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus* and *Micrococcus luteus* examined by disc diffusion method.

Experimental

Plant materials – Plants were collected from the

vicinity of Ulaanbaatar and the Khasagt Khairkhan mountain chain of the Gobi-Altai province, from June to August of 2006, respectively. Systematic identification of all plants was authenticated by Prof. Jamsran Tseden, Mongolian National University and Prof. Sanchir Chinbat, Botanical Institute of Mongolian Academy of Sciences.

Table 1. Antibacterial activity of crude ethanol extracts of some Mongolian medicinal plants

Plant species	family	extract	Inhibition zone, mm				
			Microorganisms[a]				
			E.c	*P.a*	*S.a*	*E.f*	*M.l*
Rhinantus soongoricus	Scrophulariaceae	herb	na[b]	na	na	na	9.8
Paeonia anomala	Paeoniaceae	root	10.0	na	16.5	12.0	25.7
Aquilegia sibirica	Ranunculaceae	herb	na	na	11.2	na	na
Myricaria alopecuroides	Tamaricaceae	herb	na	na	11.7	12.2	13.7
Serratula centaurica	Asteraceae	herb	na	na	na	na	10.0
Malva mohileviensis	Malvaceae	seeds	na	na	na	na	11.6
Dasiphora fruticosa	Rosaceae	flowers	na	na	11.5	10.2	12.9
Dasiphora fruticosa	Rosaceae	leaves	na	na	12.7	na	12.6
Dasiphora fruticosa	Rosaceae	stem	na	na	13.0	na	13.0
Lagotis integrifolia	Scrophulariaceae	herb	na	na	11.9	na	11.4
Comarum zalesovianum	Rosaceae	herb	na	na	24.2	15.3	24.7
Polygonum angustifolia	Polygonaceae	herb	na	na	12.0	na	12.4
Sedum hybridum	Crassulaceae	herb	na	na	10.5	17.1	14.6
Lagopsis marrubiastrum	Lamiaceae	herb	na	na	11.2	na	11.6
Cotoneaster mongolica	Rosaceae	leaves	na	na	na	na	17.5
Abies sibirica	Abiaceae	stem	na	na	13.1	na	22.0
Agrimonia pilosa	Rosaceae	herb	na	na	20.2	na	21.3
Artemisia pectinata	Compositae	herb	na	na	14.7	na	18.0
Clematis fruticosa	Ranunculaceae	herb	na	9.2	na	na	na
Dianthus superbus	Caryophyllaceae	herb	na	na	na	na	10.8
Goniolimon speciosum	Labiatae	leaves	na	na	12.9	na	10.0
Goniolimon speciosum	Labiatae	root	na	na	18.0	na	10.9
Geum alleppicum	Rosaceae	herb	na	na	na	10.0	11.2
Hedysarum alpinum	Leguminosae	herb	na	10.5	13.3	na	na
Hedysarum inundatum	Leguminosae	herb	na	8.4	na	na	na
Lomatogonum carinthiacum	Gentianaceae	herb	na	na	faint[c]	na	9.6
Pedicularis flava	Scrophulariaceae	herb	na	na	13.7	na	na
Phlomis tuberosa	Labiatae	herb	na	na	11.4	na	na
Potentilla viscosa	Rosaceae	herb	na	na	14.0	14.3	15.0
Primula farinosa	Primulaceae	herb	na	na	na	na	10.5
Pyrola incarnata	Pyrolaceae	herb	na	na	17.5	13.6	18.4
Ranunculus japanicus	Rosaceae	herb	na	na	faint	na	na
Rosa acicularis	Rosaceae	flower	na	na	13.2	12.4	15.8
Sanguisorba officinalis	Rosaceae	flower	na	na	faint	faint	13.3
Sedum aizoon	Crassulaceae	herb	na	na	13.6	9.7	11.9
Spiraea media	Rosaceae	flower	na	faint	na	na	na
Spiraea media	Rosaceae	leaves	na	faint	na	na	na
Scutellaria baicalensis	Ranunculaceae	herb	na	na	15.9	na	na

[a]**Microorganisms:** *E. c, Escherichia coli; E. f, Enterococcus faecalis; S. a, Staphylococcus aureus; M. l, Micrococcus luteus; P. a, Pseudomonas aeruginosa;*

[b]na: not active (inhibition zone was less than 0.1 mm), [c]faint: minimum active (inhibition zone was less than 1.0 mm)

Table 2. Antimicrobial activity of different fractions obtained from six plant extracts

Plant fractions		Inhibition zone, mm				
		Microorganisms[a]				
		E.c	*P.a*	*S.a*	*E.f*	*M.l*
Paeonia anomala (root)	DCM	na[b]	na	**24.1**	na	**12.5**
	ethylacetate	na	na	**23.0**	na	**16.0**
	n-butanol	na	na	na	na	**16.5**
Myricaria alopecuroides (herb)	DCM	na	na	**11.4**	na	**11.6**
	ethylacetate	na	10.5	**14.6**	na	**14.6**
	n-butanol	na	na	**12.7**	na	**11.8**
Dasiphora fruticosa (flowers)	DCM	na	na	**10.0**	na	na
	ethylacetate	faint[c]	na	**14.4**	**11.6**	**14.4**
	n-butanol	na	na	**10.8**	faint	**10.8**
	water	na	na	**10.0**	na	**11.3**
	n-hexane	na	na	**10.3**	na	faint
Dasiphora fruticosa (leaves)	DCM	na	na	na	na	na
	ethylacetate	na	na	**16.1**	na	**16.4**
	n-butanol	na	na	**10.5**	na	**11.0**
Sedum hybridum (herb)	DCM	na	na	**19.4**	na	**17.2**
	ethylacetate	na	na	**23.5**	na	**21.2**
	n-butanol	na	na	**13.0**	faint	**18.7**
	water	na	na	na	na	**9.9**
Sedum aizoon (herb)	DCM	na	na	na	na	**10.7**
	ethylacetate	na	na	**20.5**	na	**14.7**
	n-butanol	na	na	**16.3**	**10.0**	**15.4**
	water	na	na	**10.5**	na	**10.2**
Malva mohileviensis (seeds)	DCM	na	na	**11.4**	na	**13.0**
	n-butanol	na	na	**10.0**	na	faint
Standard	kanamycin	12.1	12.4	**12.6**	**10.9**	**13.7**

[a]**Microorganisms:** *E. c, Escherichia coli; E. f, Enterococcus faecalis; S. a, Staphylococcus aureus; M. l, Micrococcus luteus; P. a, Pseudomonas aeruginosa*; [b]na: not active (inhibition zone was less than 0.1 mm), [c]faint: minimum active (inhibition zone was less than 1.0 mm).

The voucher specimens have been deposited in herbariums of Natural Product Chemistry Laboratory of Institute of Chemistry and Chemical Technology, Mongolian Academy of Sciences.

Preparation of plant extracts – Each plant material was extracted exhaustively with 80% ethanol by the percolation for 3 times at RT. Each extract was filtered and concentrated under vacuum at 40 °C obtaining crude plant extracts, respectively. Some of crude ethanol extracts were suspended in water and partitioned successively with dichloromethane, ethylacetate and *n*-butanol, respectively.

Microbial strains – The test microorganisms *Escherichia coli* ATCC, *Enterococcus faecalis* ATCC, *Staphylococcus aureus* ATCC, *Micrococcus luteus* ATCC and *Pseudomonas aeruginosa* ATCC were obtained from the Microbiology Laboratory of National University of Mongolia.

Antimicrobial screening – The antimicrobial activities of plant extracts and fractions (100 mg/ml) were determined by means of the disc diffusion method (Zaidan *et al.*, 2005 and Karaman *et al.*, 2003). The standard antibiotic disc was used as kanamycin 1,000 μg/mL for *E. faecalis* and *P. aeruginosa* and 100 μg/mL for *E. coli, S. aureus* and *M. luteus*, respectively. The 80% ethanol was used as a control. One hundred mL of test organisms [10^6 colony forming units (CFU/mL)] grown in nutrient broth media for 24 h were spread over the surface of meat peptone agar medium in 9 cm diameter Petri dishes. Sterilized filter paper discs with 6 mm diameter were saturated with 50 μL with plant extracts or kanamycin per disc and they were placed on the surface of the Petri dishes. Petri dishes were incubated at 37 °C

Table 3. Minimal Inhibition Concentration (MIC) of crude ethanol extracts of the active plants

Plant species	Concentration (mg/disc)	Inhibition zone, mm		
		Microorganisms[a]		
		S.a	*E.f*	*M.l*
Sedum aizoon (herb)	4 mg/disc	**17.3**	**9.7**	**12.6**
	2 mg/disc	**13.2**	na[b]	**9.4**
	1 mg/disc	faint	na	na
	0.5 mg/disc	na	na	na
Sedum hybridum (herb)	4 mg/disc	**16.7**	**9.4**	**12.7**
	2 mg/disc	**12.6**	n.a	**8.8**
Malva mohileviensis (seeds)	4 mg/disc	**10.3**	na	**9.5**
	2 mg/disc	na	n.a	na
Dasiphora fruticosa (flowers)	4 mg/disc	**10.5**	na	**9.2**
	2 mg/disc	na	na	na
Dasiphora fruticosa (leaves)	4 mg/disc	**12.7**	na	**13.0**
	2 mg/disc	faint[c]	na	faint
Paeonia anomala (root)	4 mg/disc	**15.3**	**10.6**	**18.6**
	2 mg/disc	**12.2**	na	**16.5**
Myricaria alopecuroides (herb)	4 mg/disc	**15.1**	na	**14.3**
	2 mg/disc	**11.2**	na	**13.0**

[a]**Microorganisms:** *E. c, Escherichia coli; E. f, Enterococcus faecalis; S. a, Staphylococcus aureus; M. l, Micrococcus luteus; P. a, Pseudomonas aeruginosa*; [b]na: not active (inhibition zone was less than 0.1 mm), [c]faint: minimum active (inhibition zone was less than 1.0 mm)

for 24 h, and then the diameters of inhibition zones were measured in mm.

Determination of Minimal Inhibition Concentration (MIC) – The minimal inhibition concentration was determined as the lowest concentration that completely inhibited macroscopic growth of bacteria (Guerin-Faublee *et al.*, 1996 Tadeg *et al.*, 2005 and Rojas *et al.*, 2006). This test was performed at 6 serially diluted concentrations of each extract (0.1 mg/disc - 4 mg/disc) followed by the same modified disc diffusion method.

Results and Discussion

A total of 75 kinds of plant extracts obtained from 67 different kinds of Mongolian plant species were investigated for the antimicrobial activity by using the disc diffusion method.

The data obtained from the disc diffusion method indicated that 38 kinds of extracts prepared from 34 plant species exhibited an antimicrobial effect against some of the five tested microorganisms: *E. coli, E. faecalis, S. aureus, M. luteus* and *P. aeruginosa.* (Table 1)

Three kinds of plant extracts including the whole herb extract of *Hedysarum alpinum* exhibited a significant antimicrobial activity against *P. aeruginosa,* and 10 plant extracts including the whole herb extract of *Sedum hybridum* demonstrated a clear inhibitory zone against *E. faecalis*, respectively. (The inhibitory zone is observed over 10 mm at a concentration of 5 mg/disc). However, only one extract, the root extract of *Paeonia anomala* was revealed to exhibit an antibacterial activity against *E. coli.* Twenty four kinds of plant extracts including the whole herb extract of *Comarum zalesovianum* were found to exhibit a significant inhibition on the growth of *S. aureus* at a concentration of 5 mg/disc, and 28 plant extracts including the root extract of *P. anomala* were also found to inhibit the growth of *M. luteus* at a concentration of 5 mg/disc, respectively. (The inhibitory zone is observed over 10 mm).

The six plant extracts including the root extract of *P. anomala* which demonstrated potent antimicrobial activities at least against more than two tested microorganisms were divided into three or four solvent fractions individually which were obtained by subsequent solvent partition of corresponding plant extract with dichloromethane, ethylacetate, *n*-butanol and the resultant solvent fractions were reinvestigated for the antimicrobial activity against each microbial strain, respectively. (Table 2)

Most of the antimicrobial effect observed in plant extracts was reproduced in one or two solvent fractions

which were derived from the original plant extracts. The ethylacetate fraction and *n*-butanol fraction prepared from the whole herb extract of *S. hybridum* and *S. aizoon,* the ethylacetate fractions of leaves extract of *Dasiphora fruticosa* and that from the root extract of *P. anomala* exhibited potent antimicrobial activity against gram positive strain, *S. aureus* and *M. luteus,* respectively. While the dichloromethane fraction of seeds extract of *Malva mohileviensis* showed relatively poor activity against those two bacteria strains. The ethylacetate fraction of flowers extract of *D. fruticosa* and the *n*-butanol fraction of *S. aizoon* exhibited strong antibacterial activity against *E. faecalis.*

Most of plant extracts and derived solvent fractions exhibited poor antimicrobial activity against Gram-negative bacteria. Only the ethylacetate fraction prepared from the whole herb extract of *Myricaria alopecuroides* showed distinct antimicrobial activity against *P. aeruginosa* and the ethylacetate fraction from the flowers extract of *D. fruticosa* exhibited poor activity against *E. coli,* respectively (The inhibitory zone is observed below 2 mm).

Six kinds of plant extracts including the whole herb extract of *S. aizoon* which gave significantly potent antimicrobial activity against tested microbial strains were reinvestigated for the anitimicrobial activity at 6 serially diluted concentrations of each extract (0.1 mg/disc - 4 mg/disc) to determine MIC (Table 3). However, each plant extract did not show significant antibacterial activity at concentration of lower than 2 mg/disc. An intensive bioassay guided purification of plant extracts including the flowers extract of *D. fruticosa* and the whole herb extract of *S. hybridum* was currently conducted to identify the active constituents in the extract responsible for the antimicrobial activity.

Acknowledgments

This study was supported in part by a grant from Ministry of Science and Technology of Korea (MOST) and by Inter-Institutional Collaboration Research Program under Korea Research Council for Industrial Science & Technology (KOCI), Korea.

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(Accepted March 5, 2008)