Journal of Enzyme Inhibition and Medicinal Chemistry, June 2009; 24(3): 697–701

Antimicrobial and cytotoxicity activities of the medicinal plant *Primula macrophylla*

QAZI NAJMUS-SAQIB[1], FIAZ ALAM[1] & MANSOOR AHMAD[2]

[1]*Department of Pharmaceutical Sciences, COMSAT Institute of Information Technology, University Road, Abbotabad, NWFP, Pakistan, and* [2]*Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, University of Karachi, Karachi 75270, Pakistan*

(*Received 28 January 2008; revised 12 June 2008; accepted 1 July 2008*)

Abstract

Primula macrophylla (Primulaceae) is reported as to be useful in asthma, restlessness, insomnia and fish poisoning. Antifungal and toxic activities of crude extract, fractions and a pure isolated compound exhibited statistically significant activities. Excellent antifungal activity was found in the crude extract, benzene and ethyl acetate fractions against *T. longifusis* and against *M. canis* with different MIC values. Antileishmanial activity ($IC_{50} = 50$ug/mL) was observed as compared to standard drug Amphotericin B, and cytotoxic activity ($LD_{50} = 47.919\mu$g/mL) was also found in the chloroform fraction. While pure compound 2-phenylchromone (Flavone) isolated from the chloroform fraction showed good activity ($IC_{50} = 25\mu$g/mL) against Leishmania and cytotoxicity ($LD_{50} = 2.0116\,\mu$g/mL) in Brine Shrimp experiments. From antileishmanial and cytotoxic activity it can be concluded that 2-phenylchromone is the major compound responsible for these activities.

Keywords: Primula macrophylla, *antilieshmanial, antibacterial, antifungal, insecticidal, cytotoxic, phytotoxic, 2-phenylchromone*

Introduction

Primula macrophylla belongs to the family Primulaceae. *P. macrophylla* is very common and gregarious in stony alpine streambeds. This plant is found on K2 in the Karakorum Range. In Pakistan it is found in Shogran, Chitral, Swat, Ladak, Hazara and Kashmir valleys [1]. Usually it grows at a height of 12-16000 feet (3657.6-4876.8meters). In traditional system water extract of leaves of *P. macrophylla* is used in asthma. Tea prepared from its leaves relieves restlessness and insomnia [2]. It has antispasmodic properties. It is being used as a fish poison and has strong cytotoxic activity [3]. Other species of this genus, *P. acualis*, is reported to possess antimicrobial and anti-candida activity and this property has been proven in clinical experiments [4]. Literature review also shows that the *Primula* species besides flavonoid like Macrophylloside contain a number of saponins like Triterpenoid sapogenins, Protoprimulagenin A, priverosaponin B, Macrophyllicin etc. [5–10].

Keeping in view the traditional uses of this plant in Pakistan, it was proposed to investigate this herb for biological and chemical characterization, similar to that undertaken by M. Nisar et al. on another plant [11].

Experimental

Extract preparation

Primula macrophylla, whole fresh plant (15 kg) was collected from Kaghan valley in the month of June 2001. Plant was identified by Mr. Manzoor Hussain, Assistant Professor, Department of Botany, Postgraduate College Abbotabad. Specimen voucher of the plant material was deposited at the herbarium of the said department.

Correspondence: Q. Najmus-Saqib, Department of Environmental Sciences, COMSAT Institute of Information Technology, University Road, Abbotabad, NWFP, Pakistan. E-mail: najam48@ciit.net.pk.

ISSN 1475-6366 print/ISSN 1475-6374 online © 2009 Informa UK Ltd.
DOI: 10.1080/14756360802333406

Table I. Cytotoxic activity in crude extract and fractions *P. macrophylla*.

No.	Extract/ Fraction	LD_{50} (μg/mL)	Upper confidence limit (μg/mL)	Lower confidence limit (μg/mL)
1	Crude	1.7864	2.7164	1.0664
2	Ethyl acetate fraction	109.775	184.8888	64.8919
3	Chloroform fraction	47.919	123.2439	12.3039
4	Benzene fraction	137.556	517.7625	45.9296

Note: The aqueous methanolic fraction showed no activity.

The whole fresh plant, *Primula macrophylla,* was washed in order to remove dust or any other foreign particles. The plant was shade dried, chopped and extracted (1Kg) with distilled ethyl alcohol at room temperature for seven days, and the process was repeated twice with distilled ethyl alcohol, followed by squeezing in a muslin cloth. The combined filtrate was evaporated on a vacuum rotary evaporator under reduced pressure at 40°C. Total weight of the crude extract obtained was 64g. Later a part (60g) was fractionated with benzene, chloroform, ethyl acetate and aqueous methanol. The ethanolic extract and its different fractions were subjected to cytotoxic, phytotoxic, insecticidal, anti-leishmanial, antibacterial and antifungal assays.

Cytotoxic activity (brine shrimps bioassays)

The crude extract and its different organic fractions such as benzene (care-carcinogen), chloroform, ethyl acetate and aqueous methanol were subjected to cytotoxic bioassay. 20mg of crude plant extract and other fractions were dissolved in 2mL of respective organic solvents (benzene, chloroform, ethyl acetate and aqueous methanol) which served as stock solution. From each stock solution three different concentrations of 500, 50 and 5μg/mL were taken in vials in triplicate separately by means of micropipettes for 1000, 100 and 10ppm final concentrations (Probit analysis 'FINNEY' program) as described by Meyer et al. (1982) [12] and Dey et al. (1991) [13]. Results are given below in Table I with upper and lower confidence limits. The experiment was repeated thrice.

Antifungal activity

Crude ethanolic extract its benzene, chloroform, ethyl acetate and aqueous methanol fractions were subjected to antifungal activity. For antifungal studies, agar tube dilution assay was adopted as described the literature [14]. Six fungal cultures were used against crude extract and its fractions. The standard drug used was Miconazole. The test was repeated thrice. The results are given below in the Table II.

The fungal cultures used are as follows:

Insecticidal activity

For insecticidal activity crude extract was subjected against three species of common grain pest i.e. *Tribolium castaneum* (Red flour beetle), *Rhyzopertha dominica* (Laser grain borer) and *Callosobruchus enalis* (Pulse beetle) [14]. Results were noted after 24 h. All the insects remained alive and the samples used were found inactive. The test was repeated three times.

Table II. The antifungal activity in crude ethanolic extract and fractions of *P. macrophylla*.

	MIC (mg/mL)				
Fungus	Std. drug	Crude	Ethyl acetate	Chloroform	Benzene
Trichophyton longifusus	0.0014	0.4	0.35	–	0.39
Aspergillus flavus	0.03	0.80	–	0.90	–
Microspoum canis	0.0006	0.50	0.40	0.45	0.40
Fusarium solani	0.0012	1.20	–	–	–
Candida albicans	0.001	0.90	–	–	–
Candida glaberata	0.0005	1.50	–	–	–

Note: The aqueous methanolic fraction showed no activity. Std. drug used was Miconazole.

Moulds		
Trichophyton longifusis	(Clinical isolate)	Human pathogen
Microsporum canis	(ATCC11622)	Animal/human pathogen
Aspergillus flavus	(ATCC32611)	Human pathogen
Fusarium solani	(ATCC11712)	Plant pathogen
Yeasts		
Candida albicans	(ATCC2091)	Human pathogen
Candida glabrata	(ATCC90030)	Human pathogen

Antibacterial activity

The crude extract of *Primula macrophylla* was subjected to antibacterial activity using agar well diffusion method [14]. Reference drug used was Imipenum. The test was repeated three times but none of the samples showed any activity.

Antileishmanial activity

In this procedure crude extract its fractions and isolated compound (2-phenylchromone) were tested against *Leishmania major* [14]. Amphotericin B was used as reference drug. Chloroform fractions and the isolated compound from this fraction showed inhibitory activity, all other fractions were found inactive. The test was repeated three times. The results are presented in Table III.

Phytotoxic activity

The phytotoxic activity of crude ethanolic extract and its benzene, chloroform, ethyl acetate and aqueous methanol fractions was determined by Lemna bioassay [14]. The fronds of *Lemna minor* were used during experiments. The test was repeated three times. The results are presented in Table IV.

Isolation of cytotoxic compound

4 grams of the Chloroform fraction was chromatographed on silica gel column (Silica gel 60, E. Merck) eluted with increasing polarities of solvents and their combination. Pool C which was collected with hexane and carbon tetrachloride (8:2) was found active with $LD_{50} = 5.8399\ \mu g/mL$ (Table V).

The Pool C (300mg) was subjected to adsorption column chromatography and eluted with increasing order of polarity of solvents resulting in an active pool 'RC' with $LD_{50} = 9.8244\ \mu g/mL$ which shows that some of the activity has been lost during segregation.Tlc of this fraction in chloroform showed five compounds, which were separated with the using preparative tlc (2mm thick precoated plate E.Merck) using chloroform as solvent system. Five bands were observed under UV light, which were scratched and extracted separately. All these bands (A-E) were subjected to brine shrimps bioassays. Band 'C' which

Table III. The anti-leishmanial activity in crude extract and fractions of *P. macrophylla against Leishmania major.*

Extract/ Fraction	STD. Drug (μg/mL)	STD. % inhibition	IC_{50} of extract
Crude	0.19	100%	>100μg/mL
Chloroform fraction	0.19	100%	50μg/mL
Benzene fraction	0.19	100%	>100μg/mL
Ethyl acetate fraction	0.19	100%	>100μg/mL
2-Phenylchromone	0.19	100%	25μg/mL

Standard drug used: Amphotericin B.

Table IV. The phytotoxic activity in crude extract and fractions of *P. macrophylla.*

	No of fronds after treatment									
		Crude extract		Ethyl acetate Fraction		Chloroform Fraction		Benzene Fraction		
Conc. (μg/mL)	control	survivors	% growth regulation	survivors	% growth regulation	survivors	% growth regulation	survivors	% growth regulation
1000	22	0	100%	0	100%	0	100%	0	100%
100	22	2	90.90%	18	18.18%	0	100%	13	40.90%
10	22	15	31.18%	19	13.6%	14	36.36%	22	0%

Note: The aqueous methanolic fraction showed no activity.

Table V. Cytotoxic activity of compound, 2-phenylchromone, obtained from column chromatography of chloroform fraction of *P. macrophylla*.

Dose in μg/mL	No. of shrimps	No. of survivors	No. of dead		LD_{50} μg/mL	Upper toxic concentration μg/mL	Lower toxic concentration μg/mL
50	30	1	0	30	2.0116	2.9938	1.3027
		2	0				
		3	0				
5	30	1	1	27			
		2	2				
		3	0				
0.5	30	1	10	1			
		2	9				
		3	10				

was slightly impure showed activity ($LD_{50} = 2.0116$ μg/mL). Pure compound 2-phenylchromone, was obtained through crystalization with chloroform from band C. This compound was used in 0.5, 5 and 50 μg/mL doses for cytotoxic activity determination which showed $LD_{50} = 2.0116$. Later it was identified through, UV, IR, Mass and NMR spectroscopy as 2-phenylchromone (2-phenyl-4H-1-benzopyran-4-one) with Molecular Formula $C_{15}H_{10}O_2$ as already reported [15]

Results and discussion

The important activity tests carried out by us showed excellent results in antifungal, anti-leishmanial, cytotoxic and phytotoxic activity while no activity was observed for antibacterial and insecticidal activities.

Antifungal activity was conducted out on *T. longifusis, M. canis, Aspergillus flavus, Fusarium solani, Candida albicans, C. glabrata, T. longifusis, and M. canis.* The crude extract showed excellent activity against all the cultures used. Ethyl acetate, chloroform and benzene fraction showed no activity against *F. solani* and *candida* species. The results showed that antifungal activity was divided among the fractions and the extracts were more active against the moulds then yeasts. Among the cultures used, *M.canis* proved to be more sensitive to all the samples. While aqueous methanolic fraction exhibited no activity in this test.

Antibacterial activity was carried out on Escherichia coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi. None of the samples showed any activity.

The anti-leishmanial activity of the crude extract and fractions of *P. macrophylla* has not been reported previously. The chloroform fraction and the isolated compound (2-phenylchromone) were found active with $IC_{50} = 50$μg/mL and $IC_{50} = 25$μg/mL, respectively, against *Leishmania major* in comparison to the standard drug, Amphotericin B.

Keeping in view the cytotoxic and antileishmanial activity of chloroform fraction of *Primula macrophylla* a pure compound, 2-phenylchromone (Molecular Formula $C_{15}H_{10}O_2$.) was isolated via significant activity-directed methodology with LD_{50}(2.0116 μg/mL). This similarity shows that these activities are due to 2-phenylchromone. It is also interesting to note that there are only two functional groups in the chemical structure of this compound and it can be assumed that antifungal, antileishmanial, cytotoxic and phytotoxic activity is due to the ether and ketone groups present in the flavone.

This work indicates that this plant can be used in standardization, evaluation and development of new natural drug herbicides/weedicides. However, the toxicity of the compound on the human body is still unknown.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Nasir E, Ali SI. Flora of West Pakistan., Vol. 157 Islamabad, Pakistan: Pakistan Agriculture Research Council; 1984. p 8–10, Y.J. Nasir.
[2] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. Delhi: CSIR; 1986.
[3] Tsarong TJ. Tibetan medicinal plants. India: Tibetan Medical Publications; 1994.
[4] Von C, Margineanu V, Cucu LG, Parvu C. The anti-candida action of saponin from *Primula*. Planta Med 1976;30:35.
[5] Saqib QN, Ahmad VU, Usmanghani K. Pridentigenin E: A triterpenoid sapogenin from *P. denticulata*. Phytochemistry 1980;19:1875–1876.
[6] Saqib QN, Ahmad VU, Usmanghani K. Triterpenoid sapogenins from *Primula denticulata*. J Chem Soc Pak 1981; 1:983.
[7] Ahmad VU, Shah MG, Mohammad FV, Ismail N, Noorwala M. Macrophylloside: Flavone glucoside from *Primula macrophylla*. Phytochemistry 1991;30:4206–4208.
[8] Ahmad VU, Shah MG, Mohammad FV. Macrophyllicin: A saponin from *Primula macrophylla*. Phytochemistry 1993;32: 1543–1547.
[9] Calis I, Yuruker I. Triterpene saponins from *Primula veris* subsp. Macrocalyx and *Primula elatior* subsp. Meyeri. J Nat Prod 1992;55:1299–1306.

[10] Seims K, Jaensch M, Jakupovic J. Structures of the two saponins isolated from commercially available root extract of *Primula species*. 1998;64:272–274.

[11] Nisar M, Khan I, Ahmad B, Ali I, Ahmad W, Choudhary MI. Antifungal and antibacterial activities of *Taxus wallichiana* Zucc. J Enzyme Inhib Med Chem 2008;23(2): 256–260.

[12] Meyer BN, Ferrigni NR, Putnam JM, Jacobsen LB, Mclaughin JL. A convenient general bioassay for active plant constituents. Planta Med 1982;45:31–34.

[13] Dey PM, Harbone JB. Studies in natural products chemistry., Vol. 9 Netherland: Elsevier Science Publishers, B.V; 1991. p 383–409.

[14] Rehman A, Choudry MI, Thomson WJ. Bioassays technique for drug development. Amsterdam: Hardwood Academic Publishers; 2001.

[15] Wenkert E, Gottlieb HE. Phytochemistry 1977;16(11): 1811–1816.