Assessment of Antitumour Activity of two *Polygonum* species using Potato Disc Assay

Md. Abdul Mazid[1*], Bidyut K. Datta[2], Lutfun Nahar[3] and Satyajit D. Sarker[3]

[1]Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka 1000, Bangladesh
[2]Department of Pharmaceutical Technology, University of Dhaka, Dhaka 1000, Bangladesh
[3]School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine BT52 1SA, Co. Londonderry, Northern Ireland, UK

Abstract

Two Bangladeshi *Polygonum* species, *Polygonum barbatum* (L.) Hara *var. barbata*, common name 'bekhanjabaj' and *Polygonum stagninum* Buch.-Ham. ex Meissn., common name 'ratooti sag' or 'bara bishkatali, are perennial herbs that belong to the family Polygonaceae. As part of our on-going bioactivity and phytochemical studies on *Polygonum* species, the extracts of *P. barbatum* var. *barbata* and *P. stagninum* have been assessed for the antitumour property using the potato disc assay. All extracts showed considerable level of antitumour activity. The petroleum ether extract of *P. barbatum* var. *barbata* and the *n*-hexane and ethyl acetate extracts of *P. stagninum*, having IC_{50} values 290, 200 and 180 μg/disc, respectively, were the most active of all extracts. The methanol extracts of both plants were the least active and had an IC_{50} value >400 μg/disc. Overall, the extracts of *P. stagninum* showed better antitumour activity profiles than the extracts of *P. barbatum* var. *barbata.*

Keywords*: Polygonum barbatum* var. *barbata*; *Polygonum stagninum*; Polygonaceae; Antitumour activity; Potato disc assay.

Introduction

Polygonum barbatum (L.) Hara var. *barbata*, common name 'bekhanjabaj', and *Polygonum stagninum* Buch.-Ham. ex Meissn., common name 'ratooti sag' or 'bara bishkatali, are perennial herbs of the family Polygonaceae (Balza *et al*., 1989; GRIN Database, 2008; Kirtikar and Basu, 1999). Both species grow widely in marshy and aquatic places, by the sides of the rivers, seasonally flooded roadsides ditches and small ponds throughout Bangladesh, India and Thailand, and also in many other countries in the south-east Asia. The genus *Polygonum* is reputed for producing bioactive compounds, and also for its use in the oriental traditional medicine systems for the treatment of various ailments including fever, pain, infections, inflammation, cancer and tumour (Phytochemical and Ethnobotanical Database, 2008).

Previous phytochemical studies on *P. stagninum* revealed the presence of cinnamic acid derivatives, flavonoids and proanthocyanidin polymers (Balza *et al*., 1989; Datta *et al*., 2002); and sitosterone, viscozulenic acid and acetophenone from *P. barbatum* var. *barbata* (Mazid *et al*., 2011). Moreover, report on any bioactivity studies on these species is not available till to date (Mazid *et al*., 2010). As part of our continuing bioactivity and phytochemical studies on *Polygonum* species (Mazid *et al*. 2011; Mazid *et al*., 2010; Datta *et al*., 2000; Datta *et al*., 2001a; Datta *et al*., 2001b; Datta *et al*., 2004a; Datta *et al*., 2004b; Datta *et al*., 2007; Mazid *et al*., 2009), we report the antitumour activity of the extracts of *P. barbatum* and *P. stagninum* using the potato disc assay.

Materials and Methods

Plant materials: The aerial parts of *Polygonum barbatum* and *P. stagninum* were collected from Kajla, Rajshahi, Bangladesh and authenticated by Professor Naderuzzaman (Department of Botany, University of Rajshahi, Bangladesh). Voucher specimens, BKD2004-1 and BKD2004-2, representing this collection have been retained in the Herbarium of the Department of Botany, University of Dhaka, Dhaka, Bangladesh.

Extraction: The sun-dried and ground aerial parts of *P. barbatum* (650 g) were extracted with methanol (MeOH, 4 L) using maceration for 5 days, and sun-dried and ground aerial parts of *P. stagninum* (800 g) were

*Corresponding author: Md. Abdul Mazid, Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka 1000, Bangladesh.
E-mail: mazid_04@yahoo.com

extracted successively with *n*-hexane, ethyl acetate (EtOAc) and MeOH (4 L volume and 5 days duration for each). The extracts were concentrated by evaporation under reduced pressure at 40° C.

Solvent partitioning: The MeOH extract of *P. barbatum* was made to 90% aq. MeOH extract and subjected to solvent portioning with petroleum ether (PE). The resulting aqueous MeOH extract was further partitioned with chloroform and finally EtOAc. All solvent fractions were concentrated by evaporation under reduced pressure at 40° C.

Potato disc assay: The potato disc assay (Fegingi *et al*., 1982, McLaugghlin, 1991; McLaughlin and Rogers, 1998; Coker *et al*., 2003) was used to assess the antitumour activity of the extracts of two *Polygonum* species. *Agrobacterium tumefaciens* Cambia SR009-EHA-105, obtained from the Department of Biochemistry, University of Dhaka, Dhaka-1000, Bangladesh and cultured in King's B culture medium, was used to initiate tumour growth in fresh, disease-free and red-skinned potato discs. Dimethylsulphoxide (DMSO) was used to prepare test extracts, and appropriate volumes of test solutions were dispensed to achieve 50, 100, 200 and 400 μg/disc. More than 20% tumour inhibition was considered significant (Feringi *et al*., 1982). Data were statistically analysed by ANOVA.

Results and Discussion

The PE, $CHCl_3$, EtOAc and MeOH extracts of *P. barbatum* var. *barbata*, and the *n*-hexane, EtOAc and MeOH extracts of *P. stagninum* were assessed for antitumour activity using the potato disc assay as described in materials and methods, which is a statistically valid assay for primary screening of plants, fractions or purified compounds for possible anticancer and antitumour activity. The extracts dose-dependently inhibited the growth of gall tumour caused by *Agrobacterium tumefaciens*. The IC_{50} values as well as percent inhibition at various concentrations of the extracts are presented in Table 1.

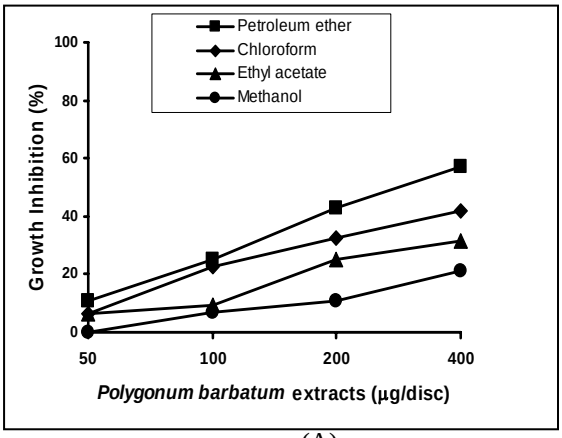
Table 1. Antitumour activity of the extracts of *Polygonum barbatum* var. *barbata* and *Polygonum stagninum*

Test extracts	% Inhibition of tumour growth at concentrations (μg/disc)				IC_{50} (μg/disc)
	50	100	200	400	
Polygonum barbatum var. *barbata*					
Petroleum ether	10.7	25.0	42.9	57.1	290
Chloroform	6.5	22.6	32.3	41.9	>400
Ethyl acetate	6.3	9.4	25.0	31.3	>400
Methanol	0.0	7.1	10.7	21.4	>400
Polygonum stagninum					
n-Hexane	16.2	30.8	50.0	69.2	200
Ethyl acetate	25.0	42.9	60.7	78.6	180
Methanol	13.3	23.3	30.0	43.3	>400

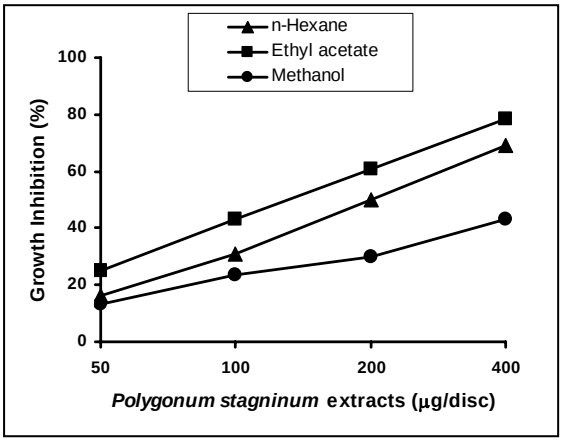
The results were compared (Figure 1) with that of the positive control, vincristine sulphate (3.125 μg/disc), which completely inhibited the growth of gall tumour on potato discs. All extracts showed considerable level of antitumour activity. The petroleum ether extract of *P. barbatum* and the *n*-hexane and ethyl acetate extracts of *P. stagninum*, having IC_{50} values 290, 200 and 180 μg/disc, respectively, were the most active of all extracts. The methanol extracts of both plants were the least active and had an IC_{50} value >400 μg/disc. Overall, the extracts of *P. stagninum* showed better antitumour activity profiles than the extracts of *P. barbatum* var. *barbata.*

The potato disc assay is an animal-sparing, fairly rapid, inexpensive and reliable bioassay that provides useful indication of anticancer and antitumour activity of test samples by the inhibition of the development of crown gall tumour on the disc of potato tubers. In fact, the inhibition of *Agrobacterium tumefaciens* induced tumours (or Crown Gall) in potato disc is an assay based on antimitotic activity and can detect a broad range of known

and novel antitumour effects (McLaughlin, 1991). This assay is based on the hypothesis that antitumour agents might inhibit the initiation and growth of tumours in both plant and animal systems, because certain tumourogenic mechanisms are similar in plants and animals (McLaughlin and Rogers, 1998). Crown gall tumour is a neoplastic disease in plants caused by a specific strain of Gram-negative bacterium, *A. tumefaciens*. The bacterium possesses large Ti (tumour inducing)-plasmids that carry genetic information (T-DNA) which upon infection transforms normal or wounded plant cells into autonomous tumour cells (Coker *et al*., 1991). The Ti-plasmid causes the plant's cells to multiply rapidly without going through apoptosis, resulting in tumour formation similar in nucleic acid content and histology to human and animal cancers (Binns and Thomashow, 1988).



(A)



(B)

Figure 1. Comparison of antitumor activities: A) extracts of *Polygonum barbatum* B) extracts of *Polygonum stagninum*

The antitumour activity demonstrated by the extracts of two *Polygonum* species in the present study is in line with the traditional uses of various *Polygonum* species in the treatment of tumours. These plants could be used as sources of potent antitumour agents for antitumour drug development.

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