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POTENTIAL ANTITUMOR ACTIVITY OF TWO *POLYGONUM* SPECIES

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Abstract –Two Bangladeshi *Polygonum* species, *P. barbatum* (L.) Hara *var. barbata* (common name 'bekhanjabaj') and *P. stagninum* Buch.-Ham. ex Meissn. (common name 'ratooti sag' or 'bara bishkatali'), are perennial herbs of the family Polygonaceae. The genus *Polygonum* L. is well-known for its use in oriental traditional medicine systems for the treatment of various ailments including fever, pain, inflammation, infections, cancer and tumors. The extracts of *P. barbatum* var. *barbata* and *P. stagninum* were assessed for potential antitumor properties using the potato disc assay. All extracts showed a considerable level of potential antitumor 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 of 290, 200 and 180 μg/disc, respectively, were the most active among the extracts. The methanol extracts of both plants were the least active and had an IC_{50} value of >400 μg/disc. Overall, the extracts of *P. stagninum* showed better antitumor activity potential than the extracts of *P. barbatum* var. *barbata.*

Key words: Polygonum barbatum var. barbata, *Polygonum stagninum*, Polygonaceae, antitumor activity, the potato disc assay

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INTRODUCTION

Polygonum barbatum (L.) Hara var. *barbata*, common name 'bekhanjabaj', and *P. 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, 2011; 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 southeast Asia. The genus *Polygonum* L. is well-known for producing bioactive compounds, and also for its use in oriental traditional medicine systems for the treatment of various ailments including fever, pain, infections, inflammation, cancer and tumors (Dr Duke's Phytochemical and Ethnobotanical Database, 2011). While 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), *P. barbatum* var. *barbata* has recently been reported to yield acetophenone, sitosterone, and a sesquit-

erpenes viscozulenic acid (Mazid et al., 2011). The analgesic, anti-inflammatory and diuretic properties of the extracts of *P. barbatum* var. *barbata* have also been documented recently (Mazid et al., 2009). However, to the best of our knowledge, there is no report on any bioactivity studies of *P. stagninum* available to date. As part of our continuing bioactivity and phytochemical studies of the *Polygonum* species (Datta et al., 2000; 2001a, b; 2002, 2004a,b; 2007; Mazid et al., 2009, 2011), we report on the potential antitumor activity of the extracts of *P. barbatum* var. *barbata* and *P. stagninum* using the potato disc assay.

MATERIALS AND METHODS

Plant materials

The aerial parts of *Polygonum barbatum* (L.) Hara var. *barbata* and *Polygonum stagninum* Buch.-Ham. ex Meissn. were collected from Kajla, Rajshahi, Bangladesh, authenticated by Professor Naderuzzaman (Department of Botany, University of Rajshahi, Bangladesh), and the voucher specimens, BKD2004-1 and BKD2004-2, respectively, representing this collection have been retained in the Herbarium of the Department of Botany, University of Dhaka, Dhaka, Bangladesh.

Extraction of plant material

The sun-dried and ground aerial parts of *P. barbatum var. barbata* (650 g) were extracted with methanol (MeOH, 4 L) using maceration for 5 days, and the sun-dried and ground aerial parts of *P. stagninum* (800 g) were extracted, successively, with *n*-hexane, ethyl acetate (EtOAc) and MeOH, 4 L each. The duration of each of these extractions was 5 days at room temperature. The extracts were concentrated by evaporation under reduced pressure at 40° C.

Fractionation: solvent partitioning

The MeOH extract of *P. barbatum* var. *barbata* was made to 90% aq. MeOH extract and subjected to solvent portioning with petroleum ether (PE). The resulting aq. MeOH extract was further partitioned with chloroform ($CHCl_3$) and finally with EtOAc. All solvent fractions were concentrated by evaporation under reduced pressure at 40° C.

The potato disc assay

The potato disc assay (Ferigni et al., 1982; McLaughlin, 1991; McLaughlin and Rogers, 1998; Coker et al., 2003) was used to assess the antitumor activity of the extracts of the 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 tumor growth in fresh, disease-free and red-skinned potato discs. Dimethyl sulfoxide (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% tumor inhibition was considered significant (Ferigni et al., 1982). Data were statistically analyzed 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 antitumor activity using the potato disc assay (Ferigni et al., 1982; McLaughlin, 1991; McLaughlin and Rogers, 1998; Coker et al., 2003), which is a well-accepted assay for the primary screening of plant extracts, fractions or purified compounds for potential anticancer and antitumor activity. The extracts dose-dependently inhibited the growth of gall tumor caused by *Agrobacterium tumefaciens*. The IC_{50} values as well as % inhibition at various concentrations of the extracts are presented in Table 1. The results were compared with that of the positive control, the well-known anticancer drug vincristine sulphate (3.125 μg/disc), which completely inhibited the growth of gall tumor on potato discs. All extracts exhibited a considerable level of antitumor activity. The PE extract of *P. barbatum* var. *barbata* and the *n*-hexane and EtOAc extracts of *P. stagni-*

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

Test extracts	% Inhibition of tumor growth at different concentrations of extracts (µg/disc)				IC_{50} (µg/disc)
	50	100	200	400	
Polygonum barbatum var. *barbata*					
Petroleum ether (PE)	10.7	25.0	42.9	57.1	290
Chloroform ($CHCl_3$)	6.5	22.6	32.3	41.9	>400
Ethyl acetate (EtOAc)	6.3	9.4	25.0	31.3	>400
Methanol (MeOH)	0.0	7.1	10.7	21.4	>400
Polygonum stagninum					
n-Hexane	16.2	30.8	50.0	69.2	200
EtOAc	25.0	42.9	60.7	78.6	180
MeOH	13.3	23.3	30.0	43.3	>400

A 100% inhibition was observed with the positive control, vincristine sulphate at a concentration of 3.125 µg/disc. There was no inhibition caused by the negative control, DMSO.

num, having IC_{50} values of 290, 200 and 180 µg/disc, respectively, were the most active of all the extracts. The MeOH extracts of both plants were the least active and had an IC_{50} value of >400 µg/disc. Overall, the extracts of *P. stagninum* showed better antitumor activity profiles than the extracts of *P. barbatum* var. *barbata*.

The inhibition of *A. tumefaciens*-induced tumors (or Crown Gall) in potato disc tissue is an assay based on antimitotic activity, and can detect a broad range of known and novel antitumor effects (McLaughlin and Rogers, 1998). It has been shown that the inhibition of Crown Gall tumor initiation on potato discs and subsequent growth showed good correlation with the compounds and extracts active in the 3PS leukemic mouse assay (Cocker et al., 2003). Ferrigni et al. (1982) demonstrated that the potato disc tumor assay was statistically more predictive of 3PS activity than either the 9KB or the 9PS cytotoxicity assays. This assay is an animal-sparing, fairly rapid, inexpensive and reliable bioassay that provides useful indication of anticancer and antitumor activity of test samples by the inhibition of the development of crown gall tumor on the disc of potato tubers. This assay is based on the hypothesis that antitumor agents might inhibit the initiation and growth of tumors in both plant and animal systems, because certain tumorogenic mechanisms are similar in plants and animals (Cocker et al., 2003). Crown gall tumor is a neoplastic disease in plants caused by a specific strain of the Gram-negative bacterium, *A. tumefaciens*. The bacterium possesses large Ti (tumor inducing) plasmids that carry genetic information (T-DNA) which upon infection transforms normal or wounded plant cells into autonomous tumor cells (Binns and Thomashow, 1988). The Ti-plasmid causes the plant's cells to multiply rapidly without going through apoptosis, resulting in tumor formation similar in nucleic acid content and histology to human and animal cancers (Coker et al., 2003).

The antitumor potential 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 tumors. These plants could be used as a source of potent antitumor agents for antitumor drug development.

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