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PHYTOCHEMICAL CHARACTERISTICS, ANTIMITOTIC, CYTOTOXIC AND ANTITUMOR ACTIVITIES OF BARK EXTRACT OF *STREBLUS ASPER* LOUR

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Key words: *Streblus asper* Lour, Secondary metabolites, Bark extract, Anticarcenogenic properties

Abstract

Ethanol extract of the stem bark of *Streblus asper* Lour was considered for qualitative assessment for its secondary metabolites content like alkaloids, glycosides, sterols and others. Bark extract revealed anticarcenogenic i.e. antimitotic, cytotoxic and antitumor activities. Results of different anticarcenogenic activities of the bark extract were discussed in relation to its secondary metabolite contents.

Introduction

Traditional medicinal plants have been recognized for their therapeutic benefits for centuries (Leonti *et al.* 2003, Gurib-Fakim 2006). However, there are still many unanswered questions about the mechanism of action of herbal drugs (Spinella 2002). *Streblus asper* Lour or Tooth brush tree of the family Moraceae is indigenous to Bangladesh (Chowdhury 1996). It is a bushy evergreen tree with milky latex and is found to grow wild all over Bangladesh. *S. asper* stem bark is traditionally used in the treatment of leprosy, piles, diarrhea, dysentery, elephantiasis and other diseases (Ghani 2003, Yusuf *et al.* 2009, Kumar *et al.* 2011). However, the systematic study for anticarcenogenic i.e. antimitotic, cytotoxic and antitumor activities of the bark extract in relation to secondary metabolites content have not been performed. Therefore, the aim of the present research was to investigate these properties of the bark extract.

Materials and Methods

Collection of bark sample: Stem bark sample was collected from a medium sized *Streblus asper* plant, grown naturally in the premises of Botanical Garden, Department of Botany, Chittagong University, Bangladesh in August, 2010. The plant was identified and a voucher specimen (accession no. 2010-88) was kept in the department. The collected bark sample (≈ 700 g) was cleaned from undesirable materials, chopped, air dried in shade at room temperature and finally ground to a coarse powder.

Extraction: About 100 g powder was macerated with ethanol (1 : 5) in a sealed container for 5 days at room temperature with occasional shaking. Extract was filtered through Whatman No.1 filter paper and evaporated to dryness under vacuum below 50ºC to get about 3 g blackish extract. The extract thus obtained was kept at 4^{o}C for future use.

Assessment of secondary metabolites: Alkaloid detecting reagents were prepared following Cromwell (1955) and assessed according to Aplin and Cannon (1971). Flavonoids, tannins and sterols were determined following Wall *et al.* (1954), Farnsworth (1966) and Bhattachrjee and Das (1969), respectively. Glycosides, saponins and resins in the extract were assessed qualitatively according to Ghani (2005).

Bioassay: Antimitotic activity of the extract was determined according to Turker and Camper (2002) using wheat seeds at germination and early seedling growth stage, cytotoxicity by brine

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shrimp nauplii lethality assay according to Meyer *et al.* (1982) and antitumor effects by assessing inhibition of tumor growth on potato disc due to *Agrobacterium tumefaciens* (Galsky *et al.* 1980). Each set of experiment was replicated three times and their mean values were taken.

Results and Discussion

Medicinal plants have curative properties due to the presence of various secondary metabolites like alkaloids, terpenoids, phenolic compounds, glycosides, steroids etc. (Savithramma *et al.* 2012). In the present work, bark extract of *S. asper* was examined qualitatively for its alkaloid content using Dragendorff's (D), Wagner's (W), Mayer's (M), Hager's (H) and Tannic acid (T) reagents. The relative abundance of different secondary metabolites contents in the extract was expressed by '+' sign in different degrees signifying the abundance. Absence of any secondary metabolite was indicated by '-' sign. Results are given in Table 1.

Table 1. Qualitative assessment of different secondary metabolites in *S. asper* stem bark.

Secondary metabolites										
Alkaloid (Reagents used)					Other secondary metabolites					
D	W	M	H	T	Flavonoid	Glycoside	Resin	Saponin	Sterol	Tannin
2+	+	+	+	2+	-	+	-	-	+	-

For alkaloid, all the five reagents gave positive result and the higher abundance was detected by Dragendorff's and Tannic acid reagents. Among other metabolites, positive responses were noted only for glycoside and sterol. The occurrence of different secondary metabolites in a medicinal plant suggests a wide range of its biological applications (Ramzi *et al*. 2008). Several alkaloids like vinblastine, vincristine, camptothecin, taxol etc. are successfully employed in cancer treatment (Syrovets and Laumonnier 2009). Glycoside derivatives also showed very promising activity in various *in vitro* and *in vivo* tests (Keller-Juslén *et al*. 1971) and two of them, ethylidene derivative, etoposide (Nakanomyo *et al*. 1986) and theylidene derivative, teniposide (van den Berg *et al.*1997), have been developed as anticancer drugs.

Inhibition of cell division is a measure of the antimitotic activity of chemical compounds. Growth inhibition test provides a way of detecting the antimitotic activity of chemical compounds under laboratory conditions.

In the present study, model of radish seed phytotoxicity assay described by Turker and Camper (2002) was used to evaluate growth stimulation or inhibition properties of the ethanol extracts of *S. asper* stem bark by taking wheat seed as test material. The seed germination counts taken on 5^{th} day following soaking were 56.00 ± 01.20 and 35.66 ± 0.88 at 1000 ppm and 7500 ppm of extract, respectively (Fig. 1). The root lengths measured after the same time intervals were 15.83 mm ± 0.34 and 10.17 mm ± 0.37 at 1000 ppm, 10,000 ppm of extract, respectively (Fig. 2). All these indicate that inhibition of the protrusion of plumule and radicle through the seed hilum at germination and the elongation of the root at seedling growth stages were due to the suppression of mitotic cell division and higher doses were more effective in inhibiting such growth activities, possibly due to high content of secondary metabolites. Antimitotic chemical compounds such as vinblastine and podophyllotoxin have been shown to inhibit cell division (Jacobs *et al.* 1981**,** White and Jacobs 1981). Several antitumour drugs such as colchicine and its analogues, podophyllotoxin and vinca alkaloids also inhibit mitosis (Biswas *et al.* 1984, Islam *et al.* 2010).

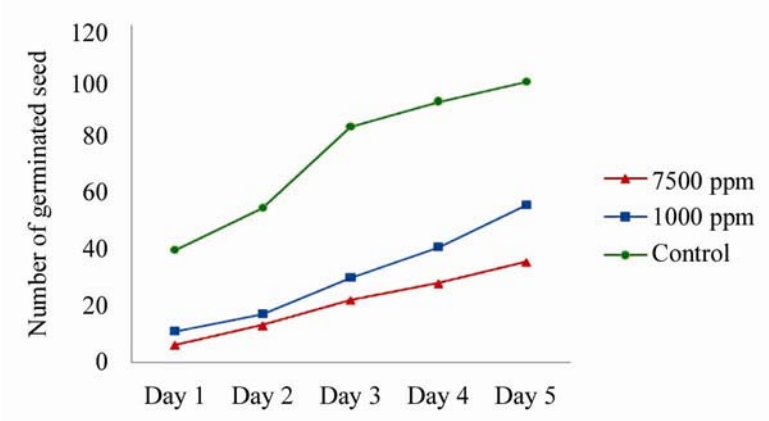

Fig. 1. Effects of Et-OH extract of *S. asper* stem bark on germination of wheat seeds.

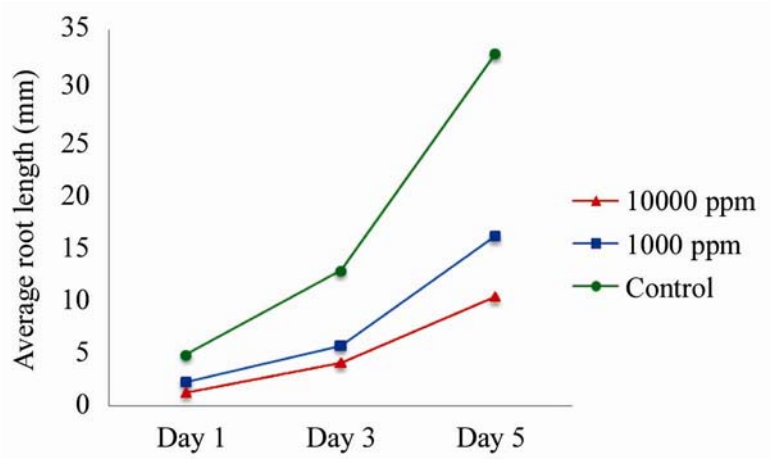

Fig. 2. Effects of Et-OH extract of *S. asper* stem bark on the rate of root growth in length of wheat seedlings.

Table 2. Cytotoxic effect on *Artemia salina* nauplii due to extract of *S. asper* stem bark.

Dose (µg/ml)	Log dose	Total nauplii	Survived nauplii	Dead nauplii	Lethality (%)	Actual (%)	Probit
10	1	10	10	0	0	0.025	3.04
20	1.3	10	9	1	10	0.1	3.72
25	1.4	10	8	2	20	0.2	4.16
50	1.7	10	6	4	40	0.4	4.75
75	1.9	10	2	8	80	0.8	5.84
100	2	10	0	10	100	0.975	6.96

The cytotoxic activity of the ethanol extract of *S. asper* stem bark extract was determined by the brine shrimp nauplii lethality bioassay using six concentrations of the extract ranging from 10 to100 µg/ml, each with 10 nauplii, which died progressively in greater number with the increase of

the concentration of the extract and exposure time (up to 24 hours) at the rate of 0.025, 0.1, 0.2, 0.4, 0.8 and 0.975% (Table 2).

In the present work, percent and probit were calculated using statistical software "Biostat 2009" and the bark extract showed LC_{50} value of 45.21 µg/ml (Table 3). Chi square value is insignificant at 5% i.e. the data between concentration and lethality is homogenous.

Table 3. Calculation of LC_{50} value, regression equation and confidence limit.

Log(LC_{50})	LC_{50} (µg/ml)	95% Confidence limit (µg/ml)	Regression equation	Chi square	
				Calculated	Tabulated
1.59	45.21	34.79-58.68	Y = –24.43 + 37.93 X	2.07	9.49

The cytotoxicity of plant material would likely indicate the presence of antitumor compounds in plant extract (Martin-Cordero *et al.* 1995). According to Rieser *et al.* (1996), crude extracts resulting an LC_{50} value of less than 250 µg/ml could be considered significantly active and potential for further investigation. The bark extract of *S. asper* exhibited LC_{50} value less than 250 µg/ml indicating its potential as a source of anticancer agent. Coker *et al.* (2003) stated that the antitumor agent might inhibit the initiation and growth of tumors in both plant and animal systems. The inhibition of *Agrobacterium tumefaciens* induced tumors i.e. crown gall, a neoplastic plant disease, 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). The crown gall tumor assay is one of several bench top bioassays recommended by the U.S. National Cancer Institute (NCI) for screening and making short list of plants with anticancer activity leading to the discovery of novel lead compounds (Lellau and Liebezeit 2003, Ahsan *et al*. 2007).

The ethanol extract of the present work exhibited the inhibitory activity in a dose dependent manner, the inhibitory effect being higher at high concentration of the extract (Fig. 3).

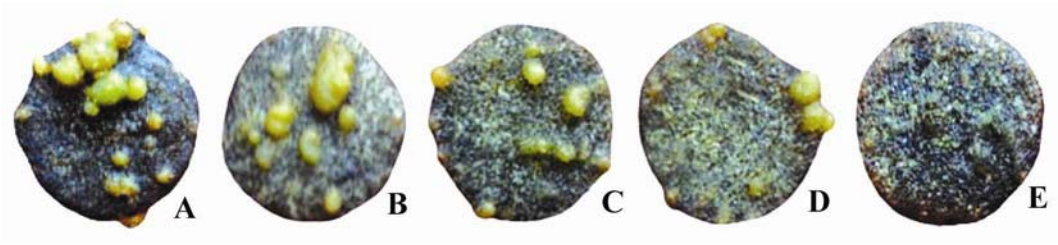

Fig. 3A-E. Effects of ethanolic extract of *S. asper* stem bark on crown gall tumor formation at different concentrations. A: negative control was without extract. B, C and D: treatment with 10, 100 and 1000 ppm extract, respectively. E: positive control with 30 ppm camptothecin.

The percent inhibition of tumor was compared with the standard tumor suppressor drug, camptothecin (positive control), which completely inhibited the growth of gall tumor on potato discs. Highly significant tumor inhibition e.g. 31.86 and 40.44% was observed at 100 ppm and 1000 ppm of the extract, respectively (Table 4). Plant extracts showing more than 20% tumor inhibition were reported to be significant (Ferrigini *et al.* 1982). Similar results were also reported by Islam *et al*. (2010) and Mazid *et al.* (2011) with other plant extracts.

Table 4. Effect of *S. asper* stem bark extract on tumor formation on potato disces.

Concentration (ppm)	Mean number of Tumor ± SE	% of Inhibition
Negative control (–extract)	22.6 ± 0.6782	-
10	18.5 ± 0.2887	18.14
100	15.4 ± 0.5099	31.86
1000	11.2 ± 0.7348	40.44
Positive control (+camptothecin)	0.00	100%

Coker *et al.* (2003) while studying the activity of antineoplastic drugs like camptothecin, paclitaxel, podophyllin, vinblastine and vincristine considered *A. tumefaciens* induced potato disc tumor assay as an effective indicator of antitumor activity.

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