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Antitumor Activity of Leaf Extracts of *Catharanthus roseus* (L.) G. Don

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Abstract

Antitumor activity of methanol leaf extracts of *Catharanthus roseus* (L.) G. Don was assayed using potato disc bioassay through *Agrobacterium tumefaciens* infection. Camptothecin used as a positive control. Significant ($P<0.05$) percentage of tumor inhibition was observed at 10ppm, 100ppm and 1000ppm of leaf extracts. Maximum tumor inhibition 80.96, 82.68 and 84.96% were observed at 1000ppm for *Agrobacterium tumefaciens* strains AtSI0105, AtAc0114 and AtTA0112, respectively. It was also observed that the strain AtSI0105 (28.06±0.29) was more dominant for producing tumor than other strains. The sensitivity test results showed that the extracts had no effect on the viability of all the tested strains of *A. tumefaciens.*

Keywords: Sensitivity test, antitumor activity, potato disc bioassay, *Agrobacterium tumefaciens*.

INTRODUCTION

Cancer is a major public health burden in both developed and developing countries (Rashed, 2014). It is a disease of misguided cells which have high potential of excess proliferation without apparent relation to the physiological demand of the process. It is world's second killer after cardiovascular disease (Kathiriya *et al*., 2010). Cancer kills about 3500 million people annually all over the world (Kaur *et al*., 2011).

Chemotherapy, radiotherapy and surgery are only three major existing modes of treatment in modern medicine for cancer. Chemotherapy is still a major challenge to the cancer patients because such highly potent drug can be toxic and less than 1% of injected drug molecules can reach their target cells, whereas the rest may damage healthy cells and tissue (Lasic, 1996). Although chemotherapy is effective in detecting cancer at a very early stage, the side effects and resistance towards drug are a major problem (Raihan *et al*., 2012). As these known methods are very costly and have side effects with limitations of their use, there is need of effective and acceptable cancer therapeutics agents that should be non-toxic, highly efficacious against multiple cancers, palatable, cost effective and acceptable by human population (Gaidhani *et al*., 2013).

Medicinal plants constitute a common alternative for cancer prevention and treatment in many countries around the world (Mehta *et al*., 2010; Desai *et al*., 2008; Guilford and Pezzuto, 2008; Soobrattee *et al*., 2006). Natural phytochemicals derived from medicinal plants have gained significant recognition in the potential management of several human clinical conditions, including cancer (Mehta *et al*., 2010; Desai *et al*., 2008; Guilford and Pezzuto, 2008). Approximately, 60% of the anticancer drugs currently used have been isolated from

natural products from the plants. More than 3000 plants worldwide have been reported to possess anticancer properties (Dai and Mumper, 2010).

Catharanthus roseus (L.) G. Don is a renowned medicinal plant. This plant is found to be rich in their pharmacological action that includes antibacterial, antifungal, antioxidant, anticancer, antiviral, anthelmintic, antidiarrheal and antidiabetic activities (Gajalakshmi *et al*., 2013; Marcone *et al*., 1997).

Different bioassays offer vast advantages for screening of medicinal plant extracts for different purposes i.e. antitumor, antibacterial, antioxidant, phytotoxic properties. Potato disc bioassay is one of them that are developed based on *Agrobacterium tumefaciens* infection on potato disc which is useful for checking antitumor properties of plant extract (Islam *et al*., 2009). The rationale for employing this bioassay rests on the fact that the tumorogenic mechanism induced by *A. tumefaciens* in plants is in many ways similar to that of animals (Becker, 1975; Braun, 1972). *Bartonella henselae* (Kempf *et al.*, 2002) and *Helicobacter pylori* (Raderer *et al*., 1998) tumor causing bacteria in human share a similar pathogenicity strategy to plant pathogen *A. tumefaciens* (Zhu *et al.*, 2000). Therefore, this study was planned to evaluate the antitumor properties of methanol leaf extracts of *C. roseus* through potato disc bioassay.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Catharanthus roseus* (L.) G. Don (Family: Apocynaceae) was collected from Rajshahi University campus during October-November, 2011. Plants were identified and authenticated by Dr. A.H.M. Mahbubur Rahman, Associate Professor and Plant taxonomist, Department of Botany, University of Rajshahi, Bangladesh.

Preparation of extracts

Collected plant materials were washed with clean sterile distilled water and dried for 3 days in oven under 60°C to reduce water content. Then the dried plant materials were crushed into fine powder using mortar-pestle and electric blender (Nokia, Osaka-Japan). Fifty gram powder was dipped into 250ml solvent (methanol) in a conical flask with rubber corks and left for two days on orbital shaking (IKA Labortechnik KS 250 Basic Orbital Shaker, Staufen, Germany). Filtration was done through teton cloth and Whatman No. 1 filter paper. The filtrate was taken into glass beaker and kept into water bath (4 holes analogue, Thermostatic water bath, China) at 60 °C for evaporation of excess solvent and stored at 4 °C (Akueshi *et al*., 2002). Then the particular concentrations i.e. 10ppm, 100ppm, and 1000ppm of the plant extracts were prepared.

Agrobacterium strains

Three *Agrobacterium tumefaciens* strains viz., AtTa0112, AtAc0114 and AtSl0105 (Islam *et al*., 2010a) were used to determine the antitumor activity of the extracts.

Bacterial culture preparation

A. tumefaciens strains were cultured on Luria-Bertani (LB) agar medium. Single colony was transferred into LB broth and incubated at 30°C for 48 hours. Six to seven loops of bacterial suspensions (1.0×10^9cfu) were transferred into test tube containing 10ml phosphate buffer (PBS; pH 7.2).

Sensitivity test of *A. tumefaciens*

Before antitumor study, sensitivity test of *A. tumefaciens* was performed to check viability of *Agrobacterium* strains using agar disc diffusion assay (Barry, 1980; Bauer *et al*., 1966). Antibiotics viz., kanamycin (30µgml^{-1}), ceprofloxacine (30µgml^{-1}) and tetracycline (30µgml^{-1}) were used as a positive control. Solvents were used as negative control. Sterilized Whatman No. 1 filter paper discs (6mm in diameter) were impregnated with 10µl of extracts (250 mgml^{-1}), antibiotics and solvents separately and followed by air dried, and then placed on seeded LB agar plates. 20µl of bacterial suspension (1.0×10^9cfu) was used for preparing seeded LB agar plates and incubated at 28°C for 24 hours. After incubation, the antibacterial activity of *C. roseus* was determined by measuring zone of inhibition against all the studied *Agrobacterium* strains.

Antitumor potato disc bioassay

Antitumor assay of plant extracts was performed according to standard potato disc bioassay (Hussain *et al*., 2007). Red skinned potatoes (*Solanum tuberosum* L. Family-Solanaceae) were collected from local market and thoroughly washed with tap and distilled water. Surface sterilization of potatoes was performed using 0.1% $HgCl_2$ solution. Potato tubers were cut into 8 mm diameter size cylindrical pieces using cork borer and transferred into sterilized distilled water (SDW) containing conical flask and washed properly. The cylindrical segments were cut into 5×8 mm size discs and placed onto agar (15gl^{-1})

plates (10 discs per plate). After that, 50µl of appropriate inoculums were placed on the surface of each potato disc and the inoculums were prepared with the mixture of 600µl test extract, 150µl SDW, 750µl *A. tumefaciens* strain in PBS. Camptothecin (30ppm) was used as positive control replacing test extracts. After inoculation, petri dishes were sealed by parafilm and incubated at 27°C for 21 days. Then the discs were stained with Lugol's solutions (10% KI, 5% I_2) and tumors were counted under a stereo microscope. The experiment was carried out in sterilized conditions and repeated three times. Percentage of tumor inhibition was calculated as described by McLaughlin and Rogers (1998). More than 20% tumor inhibition is considered significant (Ferrigni *et al*., 1982).

Statistical analysis

Data were statistically analyzed using MSTAT software (version 2.10; Russell, D. Freed, Michigan State University, USA) and expressed as mean ± SEM. Least Significant Difference (LSD) test was used to speculate further if there was a significant difference. P values <0.05 were considered as significant.

RESULTS

Sensitivity test of *A. tumefaciens*

Results showed that methanol extract of *C. roseus* has no effect on the viability of *Agrobacterium* strains because no zone of inhibition was recorded against all the studied *A. tumefaciens* strains. In contrast, inhibition zone was recorded for only studied antibiotics (positive control) and negative control did not show any visible zone of inhibition.

Antitumor potato disc bioassay

It was found that methanol leaf extract of *C. roseus* significantly (P<0.05) reduced tumor formation in a concentration dependent manner across the strains (Table 1 and 2). Significant tumor inhibition was observed at 10ppm, 100ppm and 1000ppm. Maximum 80.96, 82.68 and 84.96% and minimum 32.14, 33.32 and 45.45% tumor inhibition were recorded for *Agrobacterium* strains AtSI0105, AtAc0114 and AtTA0112, respectively (Fig. 1 and 2). It was also observed that strain AtSI0105 was more prominent for producing tumor (28.06±0.29) than other strains AtTa0112 (23.01±0.75) and AtAc0114 (25.08±0.58). Camptothecin used as a positive control and 100% tumor inhibition was observed.

Table 1: Statistical analysis of tumor inhibition by the methanol leaf extracts of *C. roseus* and tumor induction by the three strains of *A. tumefaciens* on potato discs.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F Value	Prob.
Strains (S)	2	63.72	31.86	67.11	0.000
Concentration (C)	3	1610.52	536.84	1130.80	0.000
S×C	6	70.05	11.67	24.59	
Error	22	10.44	0.47		
Total	35	1758.97			

Table 2: Antitumor activity of methanol leaf extracts of *C. roseus* and tumor induction by *A. tumefaciens* strains on potato discs.

Variable	Mean number of tumor
Strains	
AtSI0105	26.36 a
AtAc0114	23.07 b
AtTA0112	21.34 c
LSD value	0.592
Concentration	
Negative control	28.25 a
10 ppm	24.55 b
100 ppm	19.66 c
1000 ppm	12.80 d
LSD value	0.875

Means followed by different letters with the column are significantly different among the different strains and different concentrations at p<0.05.

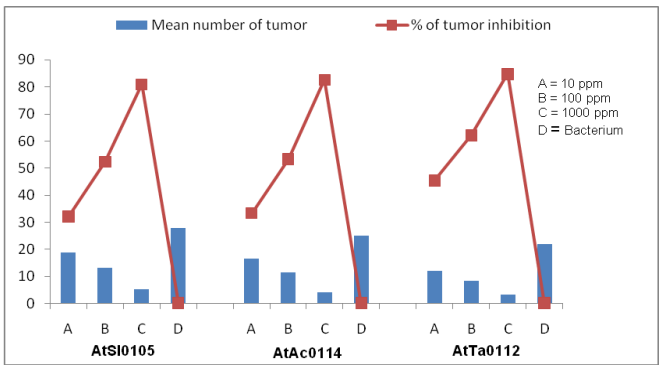

Fig. 1. Percentage of tumor inhibition by the methanol leaf extracts of *C. roseus* on potato discs at different concentrations.

Fig. 2: Photographs showing gradual tumor inhibition by the methanol leaf extract of *C. roseus* on potato discs. A_1, B_1 and C_1 as negative control; A_2, B_2 and C_2 as 10ppm plant extract; A_3, B_3 and C_3 as 100ppm plant extract; A_4, B_4 and C_4 as 1000ppm plant extract; A_5, B_5 and C_5 as 30ppm camptothecin (positive control).

DISCUSSION

From the results it reveals that *C. roseus* leaf extract had potentialities on inhibiting tumor formation. Significant percentage of tumor inhibition was observed at 10ppm, 100ppm and 1000ppm concentrations. Antitumor activity of methanol extracts increased with the increases of concentrations of the extracts indicating the efficiency as active antitumor agent. Methanol extract showed highly significant inhibition (84.86%) against the strain AtTa0112 on potato disc at 1000ppm concentration. It is well documented that alcohol (methanol) used as a solvent for plant extract preparation for their strong extraction power. However, sometimes it is often better to use alcohol (methanol) or hydroalcoholic solutions for partial lipid removal (Marston and Hostettmann, 1991). Many researchers have already been used ethanol or methanol as a solvent for evaluating cytotoxicity, phytotoxicity, antibacterial, antitumor activity in several plant species (Hussain *et al.*, 2007; Inayatullah *et al.*, 2007; Turker and Camper, 2002). Camptothecin used as a positive control and 100% tumor inhibition was observed. Similar result was observed by Turker and Camper (2002). This result may be attributed due to its DNA damaging activities. Camptothecin is a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase I (Wall *et al*., 1966).

It was shown that tumor formation was observed when *Agrobacterium* strains alive on living potato disc. The potato discs were often damaged due to the contamination and other physiological factors when there was no tumor formation. Hence successful attachment of *Agrobacteirum* on living potato disc is needed for antitumor test of plant extracts. We observed that there was no inhibitory effect of plant extract on viability of *Agrobacterium* growth. Similar result was found by Hussain *et al*. (2007) and Inayatullah *et al*. (2007). Hussain *et al*. (2007) also demonstrated antibacterial activity against *A. tumefaciens* to check whether extracts are lethal for bacteria or are inhibiting at any level that is necessary for the genetic transfer mechanism and finally induction of tumor.

Antitumor potato disc assay is a valuable tool that indicates antitumor activity of test compound by their inhibition of characteristic crown galls formation in wounded potato tissues by *A. tumefaciens* (Inayatullah *et al*., 2007). Development of a simple antitumor prescreen using a convenient and inexpensive plant tumor assay systems can offer numerous advantages as alternatives to extensive animal testing in the search for new anticancer drugs (Turker and Camper, 2002). Several scientists have used these methods over the past 15 years, and they appear to be adaptable to the purpose of standardization or quality control of bioactive compounds in such heterogeneous botanicals (Jerry and Lingling, 1998). The use of this bioassay has resulted in many short lists of plants with anti-cancer activity, and has helped with the discovery of novel compounds from plants (Islam *et al*., 2010b; Ullah *et al*., 2007).

Hence, significant percentage of tumor inhibition was occurred by the extract of *C. roseus* on potato discs. Thus, it may be conclude that the plant might be used as a potential source of antitumor agent.

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