



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading

Screening of *in vitro* cytotoxic activity of some medicinal plants used traditionally to treat cancer in Chhattisgarh state, India

Ritesh Jain, Sanmati Kumar Jain*

SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (Central University) Bilaspur, 495 009, Chhattisgarh, India

ARTICLE INFO

Article history:

Received 15 August 2011

Received in revised form 5 September 2011

Accepted 28 September 2011

Available online 15 October 2011

Keywords:

Cytotoxic

Anticancer

Chhattisgarh

MTT assay

ABSTRACT

Objective: To explore the cytotoxic activity of the alcoholic extracts of some medicinal plants used traditionally to treat cancer in Chhattisgarh state, India. **Methods:** *In-vitro* cytotoxicity of alcoholic extracts of five plants *i.e.* *Artocarpus heterophyllus*, *Alangium salvifolium*, *Buchanania lanzan*, *Sesbania grandiflora* and *Wrightia tinctoria* was studied against human breast cancer (MCF-7) and human leukemia (HL-60) tumor cell lines, using the thiazolyl blue test (MTT) assay. **Results:** Alcoholic extract of *Sesbania grandiflora* exhibited a prominent inhibitory effect against MCF-7 (IC_{50} $7.00 \pm 0.08 \mu\text{g/mL}$) and HL-60 (IC_{50} $18.50 \pm 0.60 \mu\text{g/mL}$) under *in vitro* condition. **Conclusions:** From the result it can be found that the *Sesbania grandiflora* extract has potent *in vitro* cytotoxic activity.

1. Introduction

Plants have long history of use in the treatment of cancer. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. For example, Hartwell has collected data on about 3000 plants, those of which possess anticancer properties are subsequently used as potent anticancer drugs^[1–3]. Plant secondary metabolites and their semi-synthetic derivatives continue to play an important role in anticancer drug therapy^[4,5]. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin and paclitaxel (taxol). Several promising new agents are in clinical development based on selective activity against cancer related molecular targets, including flavopiridol and combretastin A4 phosphate, and some agents which failed in earlier clinical studies are stimulating renewed interest. Sixty percent of currently used anticancer agents are derived in one way or another from natural sources^[6].

Use of plants for medicinal remedies is an integral part of the Indian cultural life and this is unlikely to change in the years to come. Many traditional healers and herbalists in the Chhattisgarh state of India have been treating cancer patients for many years using various medicinal plant species^[7,8]. Hence, an attempt has been made to screen some medicinal plants used for the prevention and treatment of cancer in Chhattisgarh state, India. It is generally known that ethnomedical data provide substantially increased chance of finding active plants relative to random approach^[9,10].

Uncontrolled proliferation is a universal property of tumor cells. Investigation of the cellular growth control mechanism has contributed to the understanding of carcinogenesis and to the identification of compounds with specific antitumoral activity^[11,12].

In this study, we have explored five alcoholic bark extracts from selected medicinal plants, *i.e.* *Artocarpus heterophyllus* (*A. heterophyllus*), *Alangium salvifolium* (*A. salvifolium*), *Buchanania lanzan* (*B. lanzan*), *Sesbania grandiflora* (*S. grandiflora*) and *Wrightia tinctoria* (*W. tinctoria*) for their cytotoxic activity on the human MCF-7 and HL-60 cell line. The selection was made on the basis of ethnobotanical information. The ethnobotanical information of the plants assayed is presented in Table 1.

*Corresponding author: Dr. Sanmati Kumar Jain, Associate Professor, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (Central University) Bilaspur, 495 009, Chhattisgarh, India.

Tel: +91-7752-260027

E-mail: cgherbsnhealth@gmail.com

Foundation Project: This work was financially supported by University Grant Commission (UGC), New Delhi, India [grant No. 10-01/2005 (SA-I)].

Table 1

Ethnobotanical information of selected medicinal plants used for treatment of cancer in Chhattisgarh.

Biological name (Family)	Local name	Part used	Mode of preparation and uses
<i>A. salvifolium</i> (L.F.) Wang. (Alangiaceae)	Ankol	Bark, roots and fruits	Bark decoction (O & T) is beneficial for the cancerous wound, and fruits are used (O) for lung cancer.
<i>A. heterophyllum</i> Lam. (Moraceae)	Kathal	Bark and roots	Root powders are used internally, and the bark is used in form of decoction (O).
<i>B. lanzan</i> Spreng. (Anacardiaceae)	Char	Seed, bark and root	Roots are used in form of powder and decoction (O), and bark powder is used with cow milk and honey (O).
<i>S. grandiflora</i> Poir. (Fabaceae)	Agasti	Bark and root	Bark and root juice is extracted. Both juices are mixed in equal proportion and given internally (O).
<i>W. tinctoria</i> (Roxb.) (Apocynaceae)	Dudhi	Bark, leaves and flowers	Bark is used in form of decoction (O). Leaves and flowers paste are applied locally (T).

O: Orally; T: Topical.

2. Materials and methods

2.1. Plants material

The bark of five plants *i.e.* *A. heterophyllum*, *A. salvifolium*, *B. lanzan*, *S. grandiflora* and *W. tinctoria* was collected during the months of November and December 2008. The plants were identified and authenticated by Dr. HB Singh, Scientist, National Institute Scientific Communication and Research (NISCAIR), New Delhi (India). The voucher specimens were stored in Herbarium of SLT Institute of Pharmaceutical Sciences, Bilaspur, India (specimen No. 01/ HSAH, 02/ HSAS, 03/ HSBL, 04/HSSG and 05/ HSWT).

2.2. Cancer cell lines

Human breast cancer (MCF-7) and human leukemia (HL-60) cell lines were provided by Deshpandey Laboratory, Bhopal, India.

2.3. Chemicals

Glutamine (Jinan Jiaquan Chemical Co, Ltd. Bombay Harbor), gentamicin (Anhui Minmentals Dev. Imp. & Exp. Co., Ltd. Japan), trypsin (Deyang Sinozyme Pharmaceutical Co., Ltd. China), non-essential amino acid (Archon Vitamin Corporation, Ievington, New Jersey), fetal calf serum (ZEN BIOTECH PVT, LTD. Hyderabad), dimethyl sulfoxide (DMSO) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT)-reagents were obtained from HIMEDIA, Mumbai, India. All other chemicals used were of analytical grade.

2.4. Preparation of extract and phytochemical screening

The dried bark of the plants was cleaned of dirt and ground to powder, using a commercial mill. Dried powder was defatted with light petrol (60–80 °C) and filtered. The residue was extracted with 90% ethyl alcohol by using Soxhlet extraction apparatus. Then solvent was completely

removed under reduced pressure and the extract was stored in vacuum desiccators. The percentage yield of the extracts was calculated. The phytochemical constituents were identified by qualitative analysis^[13].

2.5. In vitro cytotoxicity

The cytotoxic effect of five alcoholic plant extracts was evaluated by MTT assay using MCF-7 and HL-60 tumor cell lines. This MTT assay was performed according to a slight modification of the procedure reported by Mosman^[14]. Cells were cultured in minimum essential medium (MEM) supplemented with glutamine (0.6 g/L), gentamicin (25 mg/mL) and 10% fetal calf serum at 37 °C and in humidified 5% CO₂. For experiments, cells were plated in 96-well plate (10⁵ cells/ well for adherent cells or 0.3×10⁶ cells/ well for suspended cells in 100 μL of medium). After 24 h, the extracts (0.01, 0.1, 1, 10 and 100 μg/mL) dissolved in DMSO (1%) was added to each well and incubated for 96 h. The control groups received the same amount of DMSO. Doxorubicin (0.01, 0.1, 1, 10, 100 μg/mL) was used as positive control. Growth of tumoral cells was quantified by ability of living cells to reduce the yellow dye MTT to a blue formazan product. At the end of 96 h incubation, the medium in each well was replaced by fresh medium containing 0.5 mg/mL of MTT. Four hour later, the formazan product of MTT reduction was dissolved in DMSO and absorbance was measured at 550 nm. Drug effect was quantified as the percentage of control absorbance of reduced dye at 550 nm. Percentage inhibitions [100 - (absorbance of test wells/absorbance of control wells) × 100] were calculated and plotted against the concentrations used to calculate the IC₅₀ values^[15,16]. The experiments were performed in triplicate.

2.6. Statistical analysis

Data were presented as mean ± SEM. The IC₅₀ values were obtained by nonlinear regression using the GRAPHPAD program.

3. Results

Table 2

Yield of alcoholic extracts and preliminary phytochemical screening.

Plant name	Yield (%)	Alkaloids	Sterols /terpenoids	Phenolics	Coumarines	Flavanoids
<i>A. heterophyllum</i>	6.2±0.4	–	+	+	–	+
<i>A. salvifolium</i>	8.7±0.7	+	+	+	–	–
<i>B. lanzan</i>	18.5±1.1	–	+	+	–	+
<i>S. grandiflora</i>	11.6±0.6	–	+	+	+	+
<i>W. tinctoria</i>	16.6±0.8	+	+	+	–	+

+: Present; -: Absent.

Table 3Cytotoxic activity (IC₅₀ values) of plant extracts that are used in the treatment of cancer in Chhattisgarh traditional medicine (mean±SEM) (n=3).

Treatment	MCF-7 cell line		HL-60 cell line	
	IC ₅₀ μ g/mL	Status	IC ₅₀ μ g/mL	Status
<i>A. heterophyllum</i>	35.00±0.72	Moderately active	86.00±0.51	Inactive
<i>A. salvifolium</i>	97.00±0.81	Inactive	>100	Inactive
<i>B. lanzan</i>	>100	Inactive	>100	Inactive
<i>S. grandiflora</i>	7.00±0.08	Active	18.50±0.60	Active
<i>W. tinctoria</i>	10.00±0.05	Active	48.00±0.85	Moderately active

3.1. Phytochemical screening

The percentages of yielded alcoholic extract and the results of the phytochemical screening of all the plants were given in Table 2. Flavonoids, terpenoids and phenolics were identified in plants having cytotoxic activity.

3.2. In vitro cytotoxicity

In order to evaluate the cytotoxic effect of five plant extracts that are used in Chhattisgarh traditional medicine, an antiproliferative assay with two human cell lines (MCF-7 and HL-60) was performed. Table 3 showed the cytotoxic activity of the five plant extracts that are commonly used in the treatment of cancer in Chhattisgarh traditional medicine. Of the plants used to treat cancer diseases, *S. grandiflora* was active on both cell lines (IC₅₀ values 7.00±0.08 μ g/mL and 18.50±0.60 μ g/mL). *W. tinctoria* was found active on MCF-7 (IC₅₀ value 10.00±0.05 μ g/mL) and moderately active on HL-60 (IC₅₀ value 48.00±0.85 μ g/mL) cell line, while *A. heterophyllum* was found moderately active on MCF7 (IC₅₀ value 35.00±0.72 μ g/mL) and least active on HL-60 (IC₅₀ value 86.00±0.51 μ g/mL) cell line. On the other hand, *A. salvifolium* and *B. lanzan* extracts were not found active on both cell lines (IC₅₀ \geq 100 μ g/mL).

4. Discussion

In the present study, the cytotoxic effect of five alcoholic plant extracts on MCF-7 and HL-60 cells was evaluated by MTT assay. MTT assay is a well-established *in vitro* method for cytotoxicity against cancer cell lines and non-cancer cell lines[17], and here it was utilized to determine the selective

activity of the extracts. Different dilutions of extracts were treated and IC₅₀ values were calculated. In our screening program, we adopted the criteria of the American National Cancer Institute to consider a crude extract promising for further purification based on the IC₅₀ values lower than 30 μ g/mL in order to discover and develop potential anticancer natural compounds[18,19]. Cytotoxicity screening models provide important preliminary data to help selecting plant extracts with potential antineoplastic properties for future work[20,21]. It is of interest that the extract of the plants showed cytotoxicity against cancer cell line, and, if this also occurs *in vivo*, the use of these plants by traditional healer for the treatment of cancer patients would have some scientific support. Several plant species rich in flavonoids are reported having disease preventive and therapeutic properties. This observation is of particular importance since flavonoids are ingredients of many vegetables and fruits and the association of vegetable and fruit consumption with reduced cancer risk has been reported[22–24]. Cytotoxic activity recorded in the present study is in accordance with this finding, since the phytochemical evaluation indicated the presence of flavonoids in all of the three plant species with promising activity. High contents of quercetin, myricetin and kaempferol were identified in *S. grandiflora* leaf extracts[25], a novel protein fraction was isolated from the flower of *S. grandiflora* which showed potential anticancer and chemo preventive efficacy[26]. Recently nine flavonoids, artocarpin, cudraflavone C, 6–prenylapigenin, kuwanon C, norartocarpin, albanin A, cudraflavone B, brosimone I and artocarpanone were identified from the methanol extract of the wood of *A. heterophyllum* which showed *in vitro* cytotoxic activity against B16 melanoma cells[27]. The cytotoxic activities of active plants are probably due to presence of flavonoids.

From the result it shows that the *S. grandiflora* bark

extract has potent *in vitro* cytotoxic activity against both cell lines. Further studies are also in process to evaluate the most potent fraction of the active plant.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Authors are grateful to Head, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur, for providing necessary facilities. One of the authors (Mr. Ritesh Jain) is thankful to University Grant Commission (UGC), New Delhi, India for Research Fellowship [F. No.10–01/2005 (SA–I)].

References

- [1] Graham JG, Quinn ML, Fabricant DS, Farnsworth NR. Plants used against cancer—an extension of the work of Jonathan Hartwell. *J Ethnopharmacol* 2000; **73**(3): 347–377.
- [2] Cragg GM, Newman DJ. Natural product scaffolds as leads to drugs. *Future Med Chem* 2009; **1**(8): 1415–1427.
- [3] Shoeb M. Anticancer agents from medicinal plants. *Bangladesh J Pharmacol* 2006; **1**(2): 35–41.
- [4] Pan L, Chai H, Kinghorn AD. The continuing search for antitumor agents from higher plants. *Phytochem Lett* 2010; **3**(1): 1–8.
- [5] Indap MA, Radhika S, Motiwale L, Rao KVK. Quercetin: antitumor activity and pharmacological manifestations for increased therapeutic gains. *Indian J Pharm Sci* 2006; **68**: 465–469.
- [6] Cragg GM, Grothaus PG, Newman DJ. Impact of natural products on developing new anti-cancer agents. *Chem Rev* 2009; **109**(7): 3012–3043.
- [7] Traditional medicinal knowledge about herbs used in treatment of cancer in Chhattisgarh, India. Interactions with senior traditional healers. [Online] Available from: http://botanical.com/site/column_poudhia/publish/journal.cgi?folder=journal&next=11328. [Accessed on 22 October, 2009]
- [8] Jain R, Jain SK. Traditional medicinal plants as anticancer agents from Chhattisgarh, India: an overview. *Int J Phytomed* 2010; **2**(3): 186–196.
- [9] Cordell GA, Beecher CW, Pezzuto JM. Can ethnopharmacology contribute to the development of new anticancer drugs? *J Ethnopharmacol* 1991; **32**(1–3): 117–133.
- [10] Kintzios SE. Terrestrial plant derived anticancer agents and plant species used in anticancer research. *Crit Rev Plant Sci* 2006; **25**(2): 79–113.
- [11] Kang TH, Pae HO, Yoo JC, Kim NY, Kim YC, Ko GI, et al. Antiproliferative effect of alkaloids from *Sedum sarmentosum* on murine and human hepatoma cell line. *J Ethnopharmacol* 2000; **70**(2): 177–182.
- [12] Ruffa MJ, Ferraro G, Wagner ML, Calcagno ML, Campos RH, Cavallaro L. Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. *J Ethnopharmacol* 2002; **79**(3): 335–339.
- [13] Khandelwal KR. *Practical pharmacognosy, techniques and experiments*. India: Nirali Prakashan; 2006, p. 149–160.
- [14] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; **65**(1–2): 55–63.
- [15] Freshney RI. *Cytotoxicity in culture of animal cells: a manual of basic techniques*. USA: Wiley–Liss; 2000, p. 329–345.
- [16] Park JG, Lee SK, Hong IG, Kim HS, Lim KH, Choe KJ, et al. MDR1 gene expression: its effect on drug resistance to doxorubicin in human hepatocellular carcinoma cell line. *J Natl Cancer Inst* 1994; **86**(9): 700–705.
- [17] Abu–Dahab R, Afifi F. Antiproliferative activity of selected medicinal plants of Jordan against a breast adenocarcinoma cell line (MCF7). *Sci Pharm* 2007; **75**: 121–136.
- [18] Mesquita MLD, Paula JED, Pessoa C, Moraes MOD, Costa–Lotufo LV, Grougnet R, et al. Cytotoxic activity of Brazilian Cerrado plants used in traditional medicine against cancer cell lines. *J Ethnopharmacol* 2009; **123**(3): 439–445.
- [19] Steenkamp V, Gouws MC. Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer. *S Afr J Bot* 2006; **72**(4): 630–633.
- [20] Cardellina JH, Fuller RW, Gamble WR, Westergard C, Boswell J. Evolving strategies for the selection dereplication and prioritization of antitumor and HIV–inhibitory natural products extracts. In: Bohlin L, Bruhn JG. (eds.) *Bioassay methods in natural product research and development*. Dordrecht: Kluwer Academic Publisher; 1999, p. 25–36.
- [21] Baskar AA, Ignacimuthu S, Paulraj GM, Numair KSA. Chemopreventive potential of β –sitosterol in experimental colon cancer model—an *in vitro* and *in vivo* study. *BMC Complement Altern Med* 2010; **10**: 2–10.
- [22] Ramos S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. *J Nutr Biochem* 2007; **18**(7): 427–442.
- [23] Kanadaswami C, Lee L, Lee PH, Hwang J, Ke F, Huang Y, et al. The antitumor activities of flavonoids. *In Vivo* 2005; **19**(5): 895–909.
- [24] Ren W, Qiao Z, Wang H, Zhu Lei, Zhang Li. Flavonoids: promising anticancer agents. *Med Res Rev* 2003; **23**(4): 519–534.
- [25] Mustafa RA, Hamid AA, Mohammed S, Bakar FA. Total phenolic compounds flavonoids and radical scavenging activity of 21 selected tropical plants. *J Food Sci* 2010; **75**: C28–C35.
- [26] Laladhas KP, Cheriyan VT, Puliappadamba VT, Bava SV, Unnithan RG, Vijayammal PL, et al. A novel protein fraction from *Sesbania grandiflora* shows potential anticancer and chemo preventive efficacy, *in vitro* and *in vivo*. *J Cell Mol Med* 2010; **14**: 636–646.
- [27] Arung ET, Yoshikawa K, Shimizu K, Kondo R. Isoprenoid–substituted flavonoids from wood of *Artocarpus heterophyllus* on B16 melanoma cells: cytotoxicity and structural criteria. *Fitoterapia* 2010; **81**: 120–123.