Evaluation of *in vitro* Cytotoxic and Anticancer Activities of Selected Plant Extracts Growing in Bangladesh

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Cancer, also known as malignant neoplasm, is a disease characterized by uncontrolled cell growth, invasion into surrounding tissues and the potential to spread to other parts of the body. It is one of the major global health concerns, being the second leading cause of death worldwide and responsible for nearly 10 million deaths annually. Projections indicate that by 2030, 26 million new cancer cases and 17 million cancer-related deaths will occur annually. Despite many significant efforts by scientists, cancer remains a formidable threat worldwide.[1] However, cancer cell lines are used as important tools in both cancer research and drug discovery against cancers. Current treatments for cancer include chemotherapy, radiation therapy, and immunotherapy. However, despite these aggressive treatment approaches, the average death rates from cancer have not significantly improved.[2] Therefore, there is an urgent need to discover new anticancer agents that are more effective, have fewer side effects and are affordable. Plants have been used as a source of medicine since ancient times, either in the form of pure compounds or standardized extracts.[3-5] Ayurvedic therapy, a traditional Indian subcontinental medicine, has shown promising effect in the treatment of chronic diseases, including cancer. The potential use of plants as a source of new drugs remains largely untapped, with only a small fraction of plant species screened for biological activity and even fewer evaluated phytochemically or in clinical trials. Numerous plant species have been identified as potential sources of anticancer compounds, for example, *Catharanthus roseus* yields important anticancer agents such as vincristine, vinblastine, vinleurosine and vinrosidine; *Taxus brevifolia* produces taxol, while *Camptotheca acuminata* provides camptothecin.[6] Other examples include etoposide from *Podophyllum pellatum* and *Podophyllum emodi*. In Bangladesh, around 220 plant species have been identified as having anticancer properties.[7-9]

In this study, the cytotoxicity and anticancer properties of *Argyreia nervosa* (Climber, Family: Convolvulaceae), *Clerodendrum viscosum* (Herb, Family: Verbenaceae), *Moringa oleifera* (Tree, Family: Moringaceae), *Oroxylum indicum* (Tree, Family: Bignoniaceae) and *Tinospora cordifolia* (Climber, Family: Menispermaceae) were investigated against three cancer cell lines and one non-cancer cell line for the first time to develop new drug candidates for cancer treatment. The effects of *A. nervosa*, *C. viscosum*, *O. indicum* and *T. cordifolia* are reported for the first time against the Vero cell line.

Leaves of *A. nervosa*, *C. viscosum*, *M. oleifera*, root bark of *O. indicum* and stem of *T. cordifolia* were collected in August, 2019 from Sherpur district,

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Bangladesh and voucher specimens were deposited in the Dhaka University Salar Khan Herbarium (DUSH) as Suchana 01, Suchana 02, Suchana 03, Suchana 04, Suchana 05 and Suchana 06, respectively.

The selected plant parts were thoroughly washed with water to remove any dirt and impurities and then dried under sunlight until reaching an appropriate level of dryness. Once dried, the plant materials were ground into a coarse powder and sieved to achieve uniformity in texture.

To prepare the extracts, 50 g of each powdered sample was soaked in 20% aqueous methanol (700 ml). The mixtures were then left at room temperature for 72 hours with periodic gentle shaking. After that the extracts were filtered using a cotton filter followed by Whatman No. 1 filter paper and concentrated using a rotary evaporator to obtain crude extracts. The resulting crude mass was stored in airtight glass bottles at 4°C to ensure stability until use.

The anticancer activity of the plant extracts was evaluated against three cancer cell lines e.g. HeLa, BHK-21 and N4X4, and one non-cancer cell line e.g. Vero. These cell lines were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 1% penicillin-streptomycin (1:1), 0.2% gentamicin, and 10% fetal bovine serum (FBS). Cultures were maintained in a controlled environment with 5% CO_2 at 37°C. Subculturing was performed once a monolayer had formed in the flask, with cells detached using trypsin and the reaction stopped by the addition of complete medium. Then the cell lines were seeded onto a 96-well plate at specific cell concentrations (HeLa: 2×10^4 /100 µl, BHK-21: 2×10^4 /100 µl, N4X4: 1.5×10^4/ 100 µl, Vero: 1.5×10^4 /100 µl). Crude extracts at a concentration of 1 mg/mL were added to each well of the 96-well plate and the plates were incubated for 48 h at 37 °C with 5% CO_2. After incubation, cell viability was assessed using the trypan blue dye assay. Dead cells absorbed the dye and appeared blue, whereas live cells excluded the stain, facilitating enumeration of viable cells under an inverted light microscope. Duplicate assays were carried out for each sample, and cell counts were expressed as mean ± standard deviation (SD) from two independent experiments.

This study focused on evaluating the cytotoxic and anticancer properties of the extracts of leaves of *A. nervosa*, *C. viscosum* and *M. oleifera*, the root bark of *O. indicum* and stem of *T. cordifolia* on three cancer cell lines and one non-cancer cell line. The study revealed that all the extracts except *O. indicum* inhibited the 95% growth of N4X4 and Vero cells and 25 to 15% growth of HeLa cells. The root bark extract of *O. indicum* showed no activity against any tested cell lines (Table 1) (Figures 1 and 2).

Previous studies have also investigated the anticancer properties of *C. viscosum* leaves against breast carcinoma[10,11] and similar studies have been conducted with *M. oleifera*.[12] However, the effects of *A. nervosa*, *C. viscosum*, *O. indicum* and *T. cordifolia* are reported for the first time against the Vero cell line.

Table 1. The death rate of cells after treatment with plant extracts in a number of cell lines.

Plants samples	Cell death rate in % at 1 mg/ml			
	HeLa	BHK-21	N4X4	Vero
A. nervosa (extract of leaves)	25%	5%	95%	95%
C. viscosum (extract of leaves)	15%	5%	95%	95%
M. oleifera (extract of leaves)	5%	15%	95%	95%
O. indicum (extract of root bark)	5%	5%	5%	5%
T. cordifolia (extract of stem)	15%	15%	95%	95%
5% DMSO	5%	5%	5%	5%

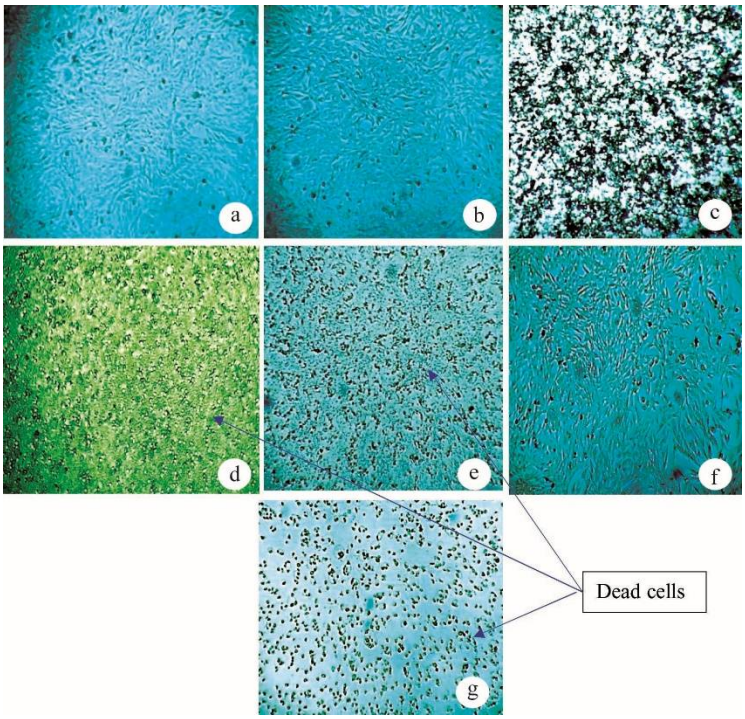

Figure 1. Morphological changes of NP-2 derivatives cell line after treatment with crude extract. a= Solvent - (untreated, without DMSO); b=Solvent + (Untreated, with DMSO); c= *A. nervosa*; d= *C. viscosum*; e=*M*. oleifera; f= *O. indicum*; g= *T. cordifolia*.

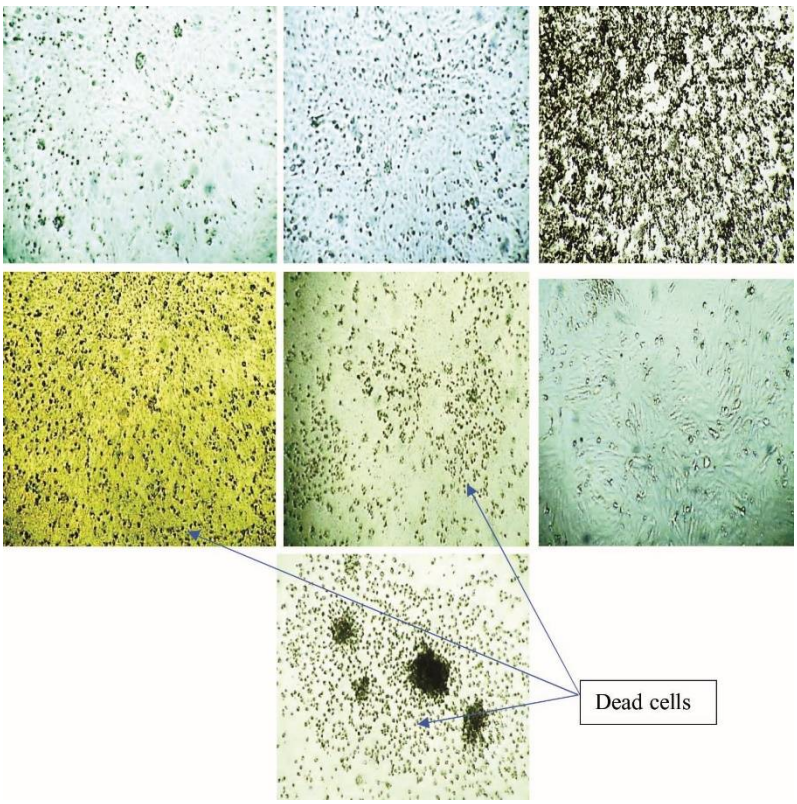

Figure 2. Morphological changes of Vero cell line after treatment with crude extracts- a= Solvent -(untreated, without DMSO); b. Solvent + (Untreated, with DMSO); c= *A. nervosa*; d= *C. viscosum*; e= *M. oleifera*; f= *O. indicum*; g= *T. cordifolia.*

The findings of this study indicate that the 20% aqueous methanolic leaf extract did not exhibit significant cytotoxicity against two cancer cell lines (HeLa and BHK-21). However, notable cytotoxicity was observed against the N4X4 cancer cell line and the non-cancer Vero cell line, with the exception of *O. indicum*. In conclusion, these results offer promising avenues for further research aimed at elucidating the active compounds and developing novel drug candidates sourced from *A. nervosa*, *C. viscosum*, *M. oleifera* and *T. cordifolia* in the treatment of glumous tumor.

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Conflict of interests

The authors declare no conflict of interest regarding this research.

REFERENCES

1. WHO (World Health Organization), International agency for research on cancer (IARC), Latest World Cancer Statistics 2013. **223**, 1-3.
2. Martin-Cordero, C., Leon-Gonzalez, A. J., Calderon-Montano, J. M., Burgos-Moron, E. and Lopez-Lazaro, M. 2012. Pro-oxidant natural products as anticancer agents. *Curr. Drug Targets* **13**, 1006-1028.
3. Cibin, T.R., Devi, D.G. and Abraham, A. 2012. Chemoprevention of two-stage skin cancer *in vivo* by *Saraca asoca*. *Integr. Cancer. Ther.* **11**, 279-286.
4. Ren, W.Y., Qiao, Z.H., Wang, H.W., Zhu, L. and Zhang, L. 2003. Flavonoids: promising anticancer agents. *Med. Res. Rev.* **23**, 519-534.
5. Gonzalez-Mejia, M.E., Voss, O.H., Murnan, E.J. and Doseff, A.I. 2010. Apigenin-induced apoptosis of leukemia cells is mediated by a bimodal and differentially regulated residue-specific phosphorylation of heat-shock protein-27. *Cell Death Dis.* **1**, 64.
6. Yusuf, M., Begum, J., Hoque, M.N. and Chowdhury, J.U. 2009. Medicinal plants of Bangladesh. Bangladesh Council of Scientific and Industrial Research Laboratories Chittagong, Bangladesh, pp. 794.
7. Sultana, N., Rahman, M.O. and Hassan, M.A. 2018. Seed germination in six anticancerous plants of Bangladesh. *J. Asiat. Soc. Bangladesh*, Sci. **45**, 151-159.
8. Anjum, A., Sultan, M. Z., Al Mansur, M. A., Hasan, C. M. and Rashid M. A. 2022. Assessment of antioxidant, antimicrobial, cytotoxicity and thrombolytic potential of *Erythrina fusca* Lour grown in Bangladesh. *Bangladesh Pharm. J.* **25**, 1-8.
9. Anjum, A., Sultan, M. Z., Hasan, C. M. and Rashid M. A. 2017. Antibacterial and cytotoxic constituents from *Bridelia verrucosa* Haines growing in Bangladesh. *Dhaka Univ. J. Pharm. Sci.* **16**, 61-68.
10. Shendge, A.K., Basu, T. and Mandal. N. 2021. Evaluation of anticancer activity of *Clerodendrum viscosum* leaves against breast carcinoma. *Ind. J. Pharmacol.* **53**, 377-383.
11. Akter, R., Uddin, S.J., Grice, I.D. and Tiralongo, E. 2014. Cytotoxic activity screening of Bangladeshi medicinal plant extracts. *J. Nat. Med.* **68**, 246-252.
12. Vasanth, K., Ilango, K., Kumar, M.K., Agarwal, A. and Dubey, G.P. 2014. Anticancer activity of *Moringa oleifera* mediated silver nanoparticles on human cervical carcinoma cells by apoptosis induction. *Colloids Surf. B Biointerfaces* **117**, 354-359.