

DRUG BIOCHEMISTRY

IN VITRO CYTOTOXICITY OF *SANCHEZIA SPECIOSA* EXTRACTS ON HUMAN EPITHELIAL CERVICAL CANCER (HELA) CELL LINENUSRAT SHAHEEN^{1*}, MUHAMMAD UZAIR, BASHIR AHMAD CH and ALAMGEER^{2*}¹Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan²Department of Pharmacology, Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan

Abstract: Cancer is one of the most distressing, life threatening disease regarding morbidity and mortality worldwide. Many folk medicinal and ornamental plants possess chemotherapeutic and chemo preventive potential. In present study, dichloromethane extract of wood, bark and root and methanol extracts of bark and root of *Sanchezia speciosa* were prepared. *Sanchezia speciosa*, an ornamental plant was evaluated for phytochemical manifestation, *in vitro* cytotoxic activity (brine shrimp lethality bioassay) and anti-cancer activity on human epithelial cervical cancer cell line (MTT assay). Preliminary phytochemical evaluation revealed the presence of alkaloids, glycosides, steroids, terpenoids and tannins. *Sanchezia speciosa* root extract in dichloromethane (DCM) showed substantial cytotoxic activity ($IC_{50} 2.528 \pm 0.31 \mu\text{g/mL}$) in brine shrimp assay and in MTT cell proliferation inhibition assay on HeLa cell line, it showed IC_{50} value of $26.7 \pm 0.72 \mu\text{g/mL}$. Effect of different concentrations of *S. speciosa* roots showed optimum cell proliferation inhibition at $100 \mu\text{g/mL}$. Thus from the results it can be deduced that *Sanchezia speciosa* extract root (DCM) possess significant cytotoxic potential in brine shrimp and MTT bioassay.

Keywords: *Sanchezia speciosa*, HeLa cell line, MTT assay, anticancer, cytotoxic activity

Cancer is uncontrolled proliferation of abnormal cells associated with genetic mutation. Despite of all considerable clinical interventions, cancer remains a devastating disease and World Health Organization (WHO) has reported death of 7.6 million people in 2008 (1) and 8.2 million in 2012 (2). Most common cancers include; lungs, breast, liver, stomach and kidneys (3). Incidence rates for cancer is twice as high in developed countries than less developed countries (4). However, mortality rate is high in less developed countries due to lack of health care facilities, poor prognosis and high cost of available treatments. Current therapeutic options for cancer are mainly chemotherapy, radiotherapy, surgery, DNA-interactive agents and hormones (5). However, these treatments are costly, painful and have severe adverse effects. Therefore, there is persistent requirement for new, affordable and effective therapeutic opportunity against cancers. Contemporary health management systems are now preferring medicinal plants for treatment of different ailments (6). In most of the developing countries indigenous medicinal plants are used as folk medicine.

Pharmacological evaluation of plants proves them a good source for the medicine development against different infections.

More than 50% of drugs available for cancer therapy are now based on plant derived natural products (7). This is a part of an effort to search new phytochemical based anti-cancer agent with more efficacy, less toxicity and cost effectiveness (8). Moreover, during various studies, phytochemicals have proved to be effective against cell proliferation, apoptosis, metastasis and angiogenic effects (9, 10). However, presently most of them are under clinical trials and only limited number of such natural product based drugs are available for cancer patients (11). In 2005, Chinese Pharmacopoeia listed that petroleum ether, ethyl acetate and methanol extracts of *B. chinensis* root exhibited significant antitumor activity in PC3, Bcap-37 and BGC-823 cells lines (12).

Ornamental plants are mainly grown for their esthetic value but many of them possess lignin, flavonoids and other medicinally important phytochemicals. Some ornamental plants are reported to

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have biological activities in various *in-vitro* and *in-vivo* studies (13). *Sanchezia speciosa* (Family: Acanthaceae), is planted as an ornamental plant. Previous reports revealed that methanol extract of *Sanchezia speciosa* leaves has shown anti-oxidant effects and significant cell growth inhibition on MCF-7 cell line (14). Ethanolic extracts of this plant possess *in-vitro* cytotoxic, anti-bacterial, anti-fungal and insecticidal activities (15). The aim of present study was to screen phytochemicals and analyze cytotoxic potential of *Sanchezia speciosa* using Brine shrimp lethality bioassay and MTT cell proliferation assay.

MATERIALS AND METHODS

Plant material

Sanchezia speciosa fresh, mature plants of 183-244 cm height, were collected in July 2015 from Multan and recognized by Dr. Zafar Ullah, Professor of Taxonomy, Department of Botany, Bahauddin Zakariya University, Multan (Voucher specimen: KEW Royal Botanic Gardens K00882617).

Preparation of plant extract

Sanchezia speciosa leaves, bark, wood and roots were shade dried and ground in mill. The pulverized plant material (700 g) was soaked in dichloromethane for 24 h; followed by methanol extraction. Extracts were concentrated by rotary evaporator under reduced pressure. Plant extracts for wood, bark and root in dichloromethane (DCM) were labeled as SSWD, SSBD and SSRD. Whereas, methanol extracts for wood, bark and root were labeled as SSWM, SSBM and SSRM, respectively.

Primary phytochemical screening

For phytochemical analysis, *Sanchezia speciosa* was subjected to recommended tests for alka-

loids, anthraquinones, glycosides, saponins, flavonoids, tannins, terpenes and steroid as described previously (16-18).

Cytotoxicity analysis

Brine shrimp lethality bioassay

Brine shrimp larvae *Artemia salina* (Leech) are often sensitive to toxic bioactive compounds. Artificial sea water was managed by adding 3.8 g sea salt in 1000 mL water and then filtered. Sea water was poured in a tank, in larger compartment shrimp eggs were added and covered with aluminum foil to darken the surrounding. Brine shrimp larvae had matured in two days. Thereafter, vials were prepared with 5 mL of sea water in each and by using pasture pipette 10 shrimps were placed in respective vial (30 shrimps/dilution) and sustained in illumination. Number of survived shrimps was examined by 3× magnifying glass, results was documented (19) and recorded as IC₅₀ value.

Hela (human epithelial cervical cancer) cell line culture

The Hela cell line was cultured in 10% fetal calf serum with 50 U/mL penicillin and 50 µg/mL streptomycin, placed in humidified incubator with 5% carbon dioxide and at 37°C temperature maintained.

MTT assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium assay was performed to access reduction of tetrazolium to formazan by production of dehydrogenase enzymes in mitochondria of proliferation cells. Hela cells were seeded (on 96 well microtiter plate) with $5 \times 10^4 - 10^5$ mL⁻¹ concentration, 200 µL per well total volume maintained and incubated at 37°C. After 24 h, fresh medium was added with different concentrations of plant extracts and control with standard anti-

Table 1. Preliminary phytochemical screening of *Sanchezia speciosa* extract.

Phytochemicals	Qualitative Screening	Result
Alkaloids	Dragendorff/ Mayer/ Wagner	+
Anthraquinone	Borntrage/ Modified Borntrager	-
Glycosides	Keller Kelliani	+
Flavonoids	Ferric Chloride/ Alkaline	-
Steroids and Terpenoids	Salkowski/ Libermann-Burchard	+
Saponins	Frothing	-
Tannins	Lead acetate /Ferric chloride	+

(+) Phytoconstituent present; (-) Phytoconstituent absent

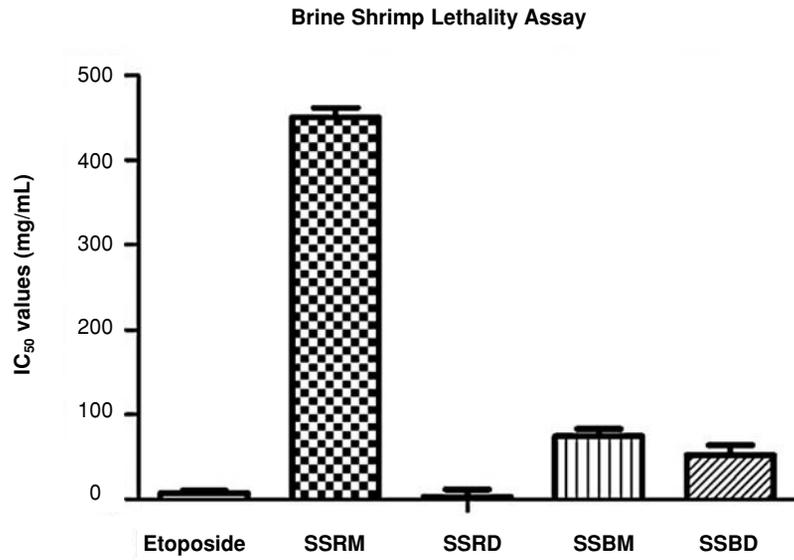


Figure 1. Cytotoxic effect of *Sanchezia speciosa* crude extracts on Brine shrimps

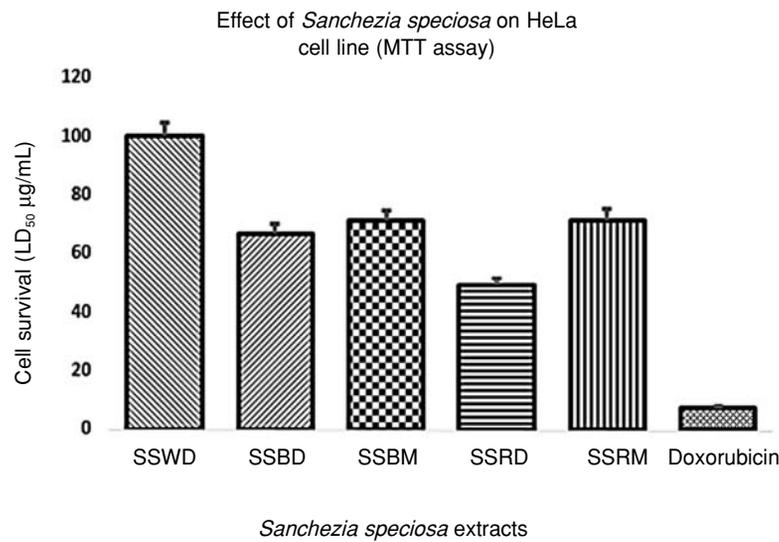


Figure 2. Hela cells survival rate after treatment with *Sanchezia speciosa* extracts

cancer drug followed incubation for 72 h at 37°C. After incubation, 15 µL of medium was replaced with 150 µL of fresh medium and MTT, respectively, and incubated for 4 h. After that, insoluble formazan was dissolved in 50 µL DMSO and absorbance was taken at 540 nm. IC₅₀ value was calculated for potent cytotoxic plant extracts against Hela cell line. Dose response of potent extract was evaluated at various concentration (6.25 µg/mL,

12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL), plates were incubated for 72 h, absorbance was measured at 540 nm by ELISA plate reader and results were recorded (20, 21).

Statistical analysis

Results were expressed as the mean ± SEM. One way Anova and T test were applied in different experiments using Graph Pad Prism 6 software.

RESULTS

Qualitative screening of *Sanchezia speciosa* for phytochemical evaluation was performed as per recommended protocols. Results illustrate the presence of alkaloids, glycosides, steroids terpenoids and tannins. On the other hand, anthraquinones, flavonoids and saponins were not detected in *Sanchezia speciosa* extract (Table 1). The cytotoxic effect of DCM and methanol extracts of *Sanchezia speciosa* bark and root were evaluated by brine shrimp lethality assay (BSLA). According to present investigation, varying degrees of lethality were observed when exposed to different doses of test samples. Moreover, lethality was found to be directly proportional to concentration of extracts tested (Fig 1). The DCM extract of *S. speciosa* root appeared to have significant cytotoxic potential with IC_{50} value of 2.52 $\mu\text{g/mL}$ comparable to standard anti-cancer drug, etoposide IC_{50} value of 7.46 $\mu\text{g/mL}$.

To evaluate the effect of *S. speciosa* on cell proliferation assay, growth prospect of cancer cells was considered in MTT assay, with bark and root extract of *S. speciosa* in DCM and methanol solvent respectively. *In-vitro* anti-cancer effect was assessed on Hela cells in 96-well plates and then incubated at 37°C at different daily intervals ($n = 3$) against absorbance of formazan at 540 nm. The number of viable cells were determined by taking doxorubicin as control with IC_{50} value calculated as 0.1 ± 0.02 $\mu\text{g/mL}$. Whereas, SSRD found to have chemotherapeutic effect with IC_{50} value 46.7 ± 1.7 $\mu\text{g/mL}$ (Fig

2). Similarly, dose response MTT assay for SSRD was performed at different concentrations indicating maximum chemotherapeutic effect at 100 $\mu\text{g/mL}$ (Fig 3).

DISCUSSION

Natural products are major source of 40% of drugs available in modern medicine system (20). Phytochemical evaluation is foremost step prior to bioactivity investigation, which leads to discovery of novel therapeutic compound. Most of the people in Pakistan rely on folk medicine from diverse flora of country. Plants contain phytochemicals which play vital role in treatment of various infections (22). Phytoconstituents such as triterpenoids, flavonoids, alkaloids and glycosides are reported to be effective in inflammation, pain, infection and cancers. In present study, phytochemical evaluation of *Sanchezia speciosa* revealed presence of alkaloids, glycosides, terpenoids, steroids and saponins (Table 1) while, alkaloids possess cytotoxic effect which is utilized for medicinal purpose (23). Previously, *Sanchezia speciosa* was explored to have significant anti-bacterial, anti-fungal and moderate insecticidal property (24). Moreover, brine shrimp lethality assay is considered as effective bioassay to assess cytotoxic effect of plant material (25, 26). In addition, this significant lethality is accredited to the occurrence of effective cytotoxic phytoconstituents (27). An extract having IC_{50} below 30 $\mu\text{g/mL}$ is generally considered as a potent bioactive extract (23). Plant

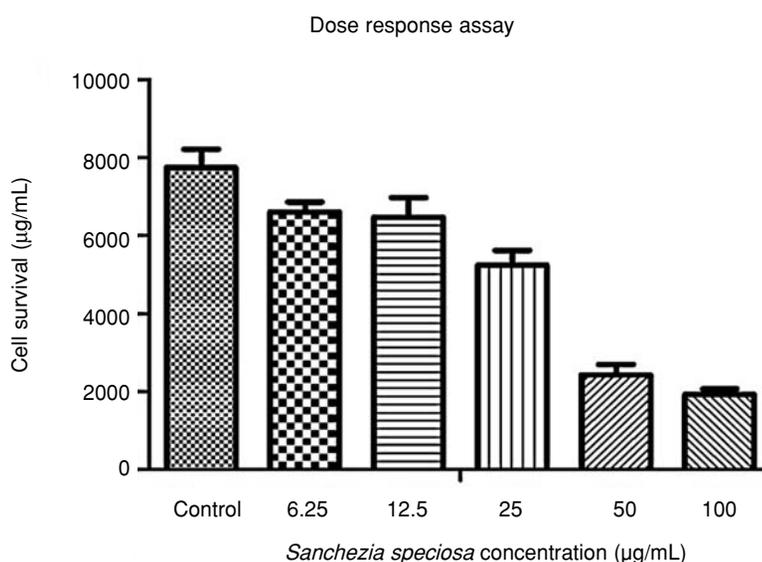


Figure 3. Hela cells survival rate at different concentrations of *Sanchezia speciosa* root (SSRD)

extracts obtained from root of *Sanchezia speciosa* (SSRD) were found to have potent cytotoxic activity (IC₅₀ value 2.52) as compared to standard anti-cancer drug etoposide (IC₅₀ value 7.46). This inhibitory effect of extract might be due to cytotoxic compounds present in extract.

MTT rapid colorimetric assay was employed to determine the effect of *Sanchezia speciosa* wood (SSWD), root (SSRM) (SSRD) and bark (SSBM) (SSBD) on Hela cells proliferation. Meanwhile, formazan product is only formed in viable respiring mitochondria of cells. To recognize *in-vitro* cell survival of Hela cells, graph was plotted between cells survived and extracts (Fig 3). The results illustrate that SSRD possess chemotherapeutic effect in the period of administration. In a previous study, methanol leaves extract of *Sanchezia speciosa* have shown anti-oxidant and anti-cancer effects (14). Whereas, no earlier study on wood, root and bark of *Sanchezia speciosa* was found. In order to investigate the effect of SSRD extract on Hela cell, another experiment was performed with serial dilutions of SSRD extract. The cell viability was significantly reduced, especially at concentration of 100 µg/mL (Fig 3). These findings suggest that *Sanchezia speciosa* root possesses cytotoxic properties due to its secondary metabolites. However, further fractionation, isolation and bioassay guided studies are indispensable to obtain pure potent anti-cancer compound.

CONCLUSION

Preliminary screening of *Sanchezia speciosa* revealed the presence of different phytochemicals. Proliferation and survival rate of Hela cells was reduced by *Sanchezia speciosa* root extract during MTT assay. Moreover, 100 µg/mL concentration of SSRD was found to be most effective in Hela cells. Therefore, *Sanchezia speciosa* root extract may possess novel potent anti-cancer compound with enhanced efficacy and limited adverse effects. Further, rigorous phytochemical mechanistic and bioassay guided studies are indispensable.

Acknowledgments

The authors highly acknowledge Faculty of Pharmacy Bahauddin Zakariya University, Multan, Pakistan for providing research facilities.

REFERENCES

1. Bray F., Ren J.S., Masuyer E., Ferlay J.: Int. J. Cancer 132, 1133 (2013).
2. Ferlay J., Soerjomataram I., Dikshit R., Eser S., Mathers C., et al.: Int. J. Cancer. 136, E359 (2015).
3. Tariq K., Rana F., Samiian L., Kilkenny J.W., Khan R., Latif N.: Clin. Adv. Hematol. Oncol. 11, 291 (2013).
4. Torre LA., Bray F., Siegel R.L., Ferlay J., Lortet-Tieulent J., Jemal A.: Cancer J. Clin. 65, 87 (2015).
5. Nussbaumer S., Bonnabry P., Veuthey J.L., Fleury-Souverain S.: Talanta 85, 2265 (2011).
6. Ullah M., Khan M.U., Mahmood A., Malik R.N., Hussain M. et al.: J. Ethnopharmacol. 150, 918 (2013).
7. Solowey E., Lichtenstein M., Sallon S., Paavilainen H., Solowey E., Lorberboum-Galski H.: Sci. World J. (2014).
8. Mothana R.A., Kriegisch S., Harms M., Wende K., Lindequist U.: Pharm. Biol. 49, 200 (2011).
9. Hadjzadeh M., Tavakol Afshari J., Ghorbani A., Shakeri M.T.: J. Med. Plants 2, 41 (2006).
10. Sadeghnia H.R., Ghorbani Hesari T., Mortazavian S.M., Mousavi S.H., Tayarani-Najaran Z., Ghorbani A.: BioMed. Res. Int. (2014).
11. Tan W., Lu J., Huang M., Li Y., Chen M. et al.: Chin. Med. 6, 27 (2011).
12. Liu M., Yang S., Jin L., Hu D., Wu Z., Yang S.: Molecules 17, 6156 (2012).
13. Koyama J., Morita I., Kobayashi N., Hirai K., Simamura E. et al.: Biol. Pharm. Bull. 29, 2310 (2006).
14. Paydar M., Wong Y.L., Moharam B.A., Wong W.F., Looi C.Y.: Pak. J. Biol. Sci. 16, 1212 (2013).
15. Parvin S., Rafshanjani M.A.S., Kader M.A., Sharmin T.: Int. J. Adv. Sci. Res. 1, 145 (2015).
16. Brain K.R., Turner T.D.: Practical evaluation of phytopharmaceuticals, 1st edn., Wright-Scientifica, Bristol 1975.
17. Evans W.C.: Trease and Evans' pharmacognosy, 16 edn., Elsevier Health Sciences 2009.
18. Raaman N.: Phytochemical techniques. 1st edn. New India Publishing agency, New Delhi 2006.
19. Choudhary M.I., Thomsen W.J.: Taylor & Francis, (2001).
20. Gomme PT., McCann K.B., Bertolini J.: Drug. Discov. Today 10, 267 (2005).
21. Huebers H.A., Finch C.A.: Physiol. Rev. 67, 520 (1987).
22. Zhuang S.R., Chen S.L., Tsai J.H., Huang C.C., Wu T.C. et al.: Phytother. Res. 23: 785 (2009).
23. McLaughlin J.L., Chang C.J., Smith D.: Stud. Nat. Prod. Chem. 9, 383 (1991).
24. Rafshanjani M., Parvin S., Kader M., Saha M.R. A.: Int. Res. J. Pharm. 5, 717 (2014).

25. Pelka M., Danzl C., Distler W., Petschelt A.: J. Dent. 28, 341 (2000).
26. Sleet R., Brendel K.: Ecotoxicol. Environ. Saf. 7, 435 (1983).
27. Siqueira J.M.D., Bomm MD., Pereira N.F.G., Garcez W.S., Boaventura M.A.D.: Quím. Nova 21, 557 (1998).

Received: 14. 08. 2016