IN VITRO ANTICANCER SCREENING OF *ASPARAGUS LARICINUS* EXTRACTS

S. Mashele and N. Kolesnikova

Faculty of Health and Environmental Studies, Central University of Technology, Free State, Private Bag X20539, Bloemfontein, RSA
Bioprospecting, Biosciences, CSIR, PO Box 395, Pretoria, 0001, RSA

Summary

The aim of the present study was to determine the anticancer activity of *Asparagus laricinus* extracts. Alcoholic and aqueous extracts were tested for their growth inhibitory effects in vitro against three human cancer cell lines: breast cancer cells, MCF7; renal cancer cells, TK10 and melanoma, UACC62 using the Sulforhodamine B (SRB) assay. Extracts were classified into four categories based on their total growth inhibition of the cell lines. Extracts which exhibited a total growth inhibition (TGI) of less than 6.25 µg/mL were regarded as potent. Alcoholic extract of *Asparagus laricinus* exhibited pronounced activity especially against the melanoma cell line UACC-62. The aqueous extract was classified as weakly active. So evaluation of *Asparagus laricinus* in the prevention and treatment of cancer is recommended. The isolation of active ingredients from these extracts is suggested.

Key words: *Asparagus laricinus*, cancer screening

Corresponding author:

Professor SS Mashele

School of Health Technology

Faculty of Health and Environmental Sciences

Central University of Technology, Free State

Private Bag X20539

Bloemfontein

South Africa

E-mail: smashele@cut.ac.za

Introduction

Cancer is one of the most prominent diseases in humans and currently there is considerable scientific and commercial interest in the continuing discovery of new anti-cancer agents from natural product sources (1). The potential of using natural products as anti-cancer agents was recognized in the 1950s by the U.S. National Cancer Institute (NCI) and has since made major contributions to the discovery of new naturally occurring anti-cancer agents (2).

Currently, over 50% of drugs used in clinical trials for anti-cancer activity were isolated from natural sources or are related to them (3). Hence, the search for natural products to be used in cancer therapy represents an area of great interest in which the plant kingdom has been the most important source, providing many anti-tumor agents with novel structures and unique mechanisms of action (4).

The study was prompted by case reports describing unexpected improvement of patients who have been terminally ill due to advanced prostate cancer. On the clinician inquiry, these patients reported that they had been treated with an extract from a root of a medicinal plant. This plant material was offered to us for an initial analysis for anti-cancer activity. The surprisingly high antimutagenic activity prompted a broad experimental investigation. The source of the plant root was identified as belonging to Asparagaceae. This is a monogeneric family (5), which was previously included within the Liliaceae. The genus *Asparagus* comprises approximately 100 species and consists of herbs, shrubs and vines. To date we have demonstrated the antimutagenic effect of *Asparagus laricinus* root extract using the Ames test (6). We believe further investigation could result in the development of new anti-cancer drugs

The aim of the present study was to identify the anticancer activity of *Asparagus* l*aricinus* against three human cell lines namely, breast MCF7, renal TK10 and melanoma UACC62. These cell lines were selected because of their high sensitivity to detect anticancer activity. We demonstrated here that these extracts exhibit anticancer activity against the three human cell lines.

Materials and Methods

Plant material

The plant material (*Asparagus laricinus*) was authenticated by scientists at the National Botanical Gardens in Pretoria South Africa. The collected materials were dried at room temperature and pulverized by mechanical mills and weighed. It was then stored in a cool place until analysis.

Extraction methods

Plant material (10g of the dried roots) were weighed, pulverized and soaked with two different solvents, namely ethanol and purified water for 72 hours with occasional stirring. The extracts were filtered and the solvent was removed completely by rotator evaporator.

In vitro anticancer screening [Council of Scientific and Industrial Research (CSIR) and National Cancer Institute (NCI) in the USA]

The human cell lines TK10, UACC62 and MCF7 were obtained from NCI in the framework of a collaborative research program between CSIR and NCI. The extracts and compounds were assayed in the 3-cell line panel consisting of TK10 (renal), MCF7 (breast), and UACC62 (melanoma). Cell lines were routinely maintained as monolayer cell cultures at 37 °C, 5% CO_2 and 100% relative humidity in RPMI containing 5% fetal bovine serum, 2mM L-glutamate and 50µg/ml gentamicin. The primary anticancer assay was performed at the CSIR in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute (7,8,9). The extracts or compounds were tested at a single concentration (100 ppm) and the culture was incubated for 48 h. End point determinations were made with a protein-binding dye, Sulforhodamine B (SRB). The growth percentage was evaluated spectrophotometrically versus controls not treated with test agents. Results for each extract were reported as the growth percentage of the treated cells, compared to that of the untreated control cells. All the extracts which reduced the growth of two of the cell lines by 75% or more, were further tested at 1/2 log serial dilutions of five concentrations ranging from 6.25-100 ppm. Cells without drug addition served as controls. The blanks contain complete medium without cells. Etoposide was used as a standard. Results of five dose screening were reported as TGI (total growth inhibition). The biological activities were separated into 4 categories: inactive (TGI >50 ppm), weak activity (15 ppm< TGI <50 ppm), moderate activity (6.25 ppm< TGI <15 ppm) and potent activity (TGI <6.25 ppm).

Results

The results of the five-dose screening are reported as TGI. The biological activities were separated into 4 categories: inactive (TGI >50 ppm), weak activity (15 ppm< TGI <50 ppm), moderate activity (6.25 ppm< TGI <15 ppm) and potent activity (TGI <6.25 ppm).

For each tested extract, three additional parameters were calculated: GI_{50} (50% growth inhibition, as opposed to TGI which indicates 100% growth inhibition – indicates the cytostatic activity the test agent), LC_{50} (50% lethal concentration - indicates the cytotoxic effect of the test agent), LC_{100} (100% lethal concentration).

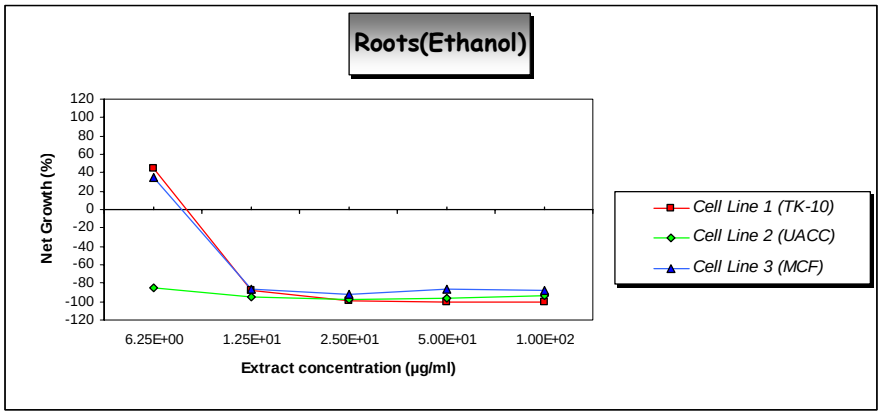

Figure 1. Growth inhibitory effect of ethanol extracts of *Asparagus laricinus* on three human cancer cell lines: MCF7; TK10 and UACC62.

Table 1. GI_{50}, TGI, LC_{50} and LC_{100} of ethanol extract of *Asparagus laricinus* on three human cell lines: MCF7, TK10 and UACC62

Activities	TK-10	UACC-62	MCF-7
GI_{50}	<6.25	<6.25	<6.25
TGI	8.34	<6.25	8.04
LC_{50}	10.70	<6.25	10.62
LC_{100}	45.00	<6.25	<6.25

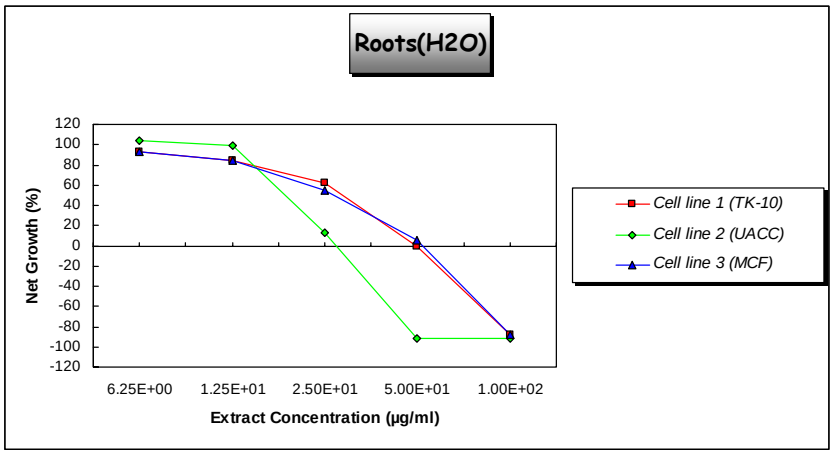

Figure 2. Growth inhibitory effect of aqueous extracts of *Asparagus laricinus* on three human cancer cell lines: MCF7; TK10 and UACC62.

Table 2. GI_{50}, TGI, LC_{50} and LC_{100} of aqueous extract of *Asparagus laricinus* on three human cell lines: MCF7, TK10 and UACC62

Activities	TK-10	UACC-62	MCF-7
GI_{50}	29.70	19.59	27.25
TGI	49.98	27.99	52.83
LC_{50}	78.39	40.02	79.57
LC_{100}	>100	>100	>100

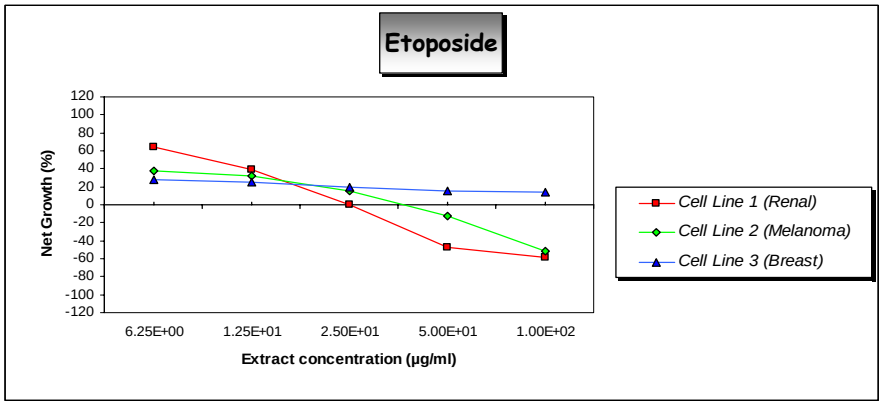

Figure 3. Growth inhibitory effect of Etoposide on three human cancer cell lines: MCF7; TK10 and UACC62.

Table 3. GI_{50}, TGI, LC_{50} and LC_{100} of Etoposide on three human cell lines: MCF7, TK10 and UACC62

Activities	**TK-10**	**UACC-62**	**MCF-7**
GI_{50}	**9.72**	**<6.25**	**<6.25**
TGI	**25.19**	**38.54**	**>100**
LC_{50}	**62.54**	**98.08**	**>100**
LC_{100}	**>100**	**>100**	**>100**

Table 4. CSIR Criteria

TGI	Status
> 50ppm	Inactive
< 50ppm >15ppm	Weak Activity
< 15ppm > 6.25ppm	Moderate Activity
< 6.25ppm	Potent Activity

Table 5. Results Summary

Extract	TGI, ppm TK-10	TGI,ppm UACC-62	TGI, ppm MCF-7
Roots(Ethanol)	**8.34**	**<6.25**	**8.04**
Roots(H2O)	**49.98**	**27.99**	**52.83**
Etoposide	**25.19**	**38.64**	**>100**

According to CSIR criteria, the Roots (Ethanol) extract can be estimated as very active, especially against the melanoma cell line UACC-62. The Roots (H_2O) extract would be classified as Weakly Active.

Discussion

A significant part of drug discovery in the last forty years has been focused on agents to prevent or treat cancer. This is not surprising because, in most developed countries and, to a greater extent, in developing countries, cancer is amongst the three most common causes of death and morbidity (10). Most of the cancer drugs in clinical use have been derived from plants. We found no literature where the cytotoxic activity of *Asparagus laricinus* has previously been investigated. To date we have demonstrated the antimutagenic effect of *Asparagus laricinus* using the Ames test (6).

According to CSIR criteria shown in Table 4, the ethanol extract of *Asparagus laricinus* can be estimated as very active (Figure 1 and Table 1), especially against the melanoma cell line UACC-62. The aqueous extract of *Asparagus laricinus* would be classified as Weakly Active (Figure 2 and Table 2). The ethanol extract is more active than Etopoxide (Figure 3 and Table 3). From the summary of the results (Table 5), it can be concluded that ethanol extract of *Asparagus laricinus* was active against the three human cell lines. This effect has stimulated us to carry out ongoing work to determine the active ingredients since these may prove valuable lead compounds in the light of their ability to kill cancer cells. No data relative to cytotoxic components of *Asparagus laricinus* had been reported and further phytochemical work on the isolation and identification of active structures of this plant is in progress.

Acknowledgements

The authors would like to thank the National Botanical Gardens for the identification of plant specimens and the Council of Scientific and Industrial Research (CSIR) for performing the SRB assays of samples.

References

1. Kinghorn, A.D., Farnsworth, N.R., Soejarto, D.D., Codell, G.A. (2003) Novel strategies for the discovery of plant-derived anti-cancer agents. *Pharmaceutical Biology* 41, 53-67.
2. Cragg, G.M., Newman, D.J. (2005) Plants as a source of anti-cancer agents. *Journal of Ethnopharmacology* 100, 72-79.
3. Newman, D.J., Cragg, G.M. (2007) natural products as source of new drugs over the last 25 years. Journal of Natural Products 70, 461-477.
4. Chang, C., Ashendel, C., Chan, T.C.K., Geahlen, R.L., McLaughlin, J., Waters, D.j. (1999) Oncogene signal transduction inhibitors from Chinese medicinal plants. *Pure Applied Chemistry* 71, 1101-1104.
5. Brummitt, R.K. (1992) Vascular plant families and genera. Royal Botanical Gardens, Kew, 804
6. Mashele, S.S. (2008) Use of *in vitro* assays to assess the antimutagenic, mutagenic and cytotoxic effect of Asparagus laricinus plant associated with the prevention and treatment of prostate cancer. *Advances in Pharmacology and Toxicology* 9 (2), 51-55.
7. Monks A, Scudiero D, Skehan P, et al. Feasibility of a High-Flux Anticancer Drug Screen using a Diverse Panel of Cultured Human Tumor Cell Lines. *Journal of the National Cancer Institute* 1991; 83: 757–766.
8. Kuo SC, Lee HZ., Juang JP, et al. Synthesis and cytotoxicity of 1,6,7,8-substituted 2-(4'-substituted phenyl)-4-quinolones and related compounds: Identification as antimitotic agents interacting with tubulin. *J Med Chem* 1993; 36: 1146–1156.
9. Leteurtre F, Kohlhagen G, Paull KD, Pommier Y. Topoisomerase II inhibition and cytotoxicity of the anthrapyrazoles DuP 937 and DuP 941 (Losoxantrone) in the National Cancer Institute preclinical antitumor drug discovery screen. *Journal of the National Cancer Institute*. 1994; 86: 1239–1244.
10. Houghton, P., Fang R., Techatanawat, I., Stevenson, G., Hylands, P.J., Lee, C.C. (2007) The SRB assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anti-cancer activity. *Methods* 42, 377-387.