The Pharma Innovation Journal 2021; 10(2): 355-358

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2021; 10(2): 355-358

www.thepharmajournal.com
Received: 25-12-2020
Accepted: 27-01-2021

G Dhanusha
MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Tamil Nadu, India

S Sujith
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Tamil Nadu, India

AR Nisha
Assistant Professor and Head, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Tamil Nadu, India

KK Aathira
MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Tamil Nadu, India

JS Haima
MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Tamil Nadu, India

Corresponding Author:
S Sujith
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Tamil Nadu, India

Cytotoxic and antiproliferative potential of methanolic extracts of *Asparagus racemosus* in MDAMB231 cells

G Dhanusha, S Sujith, AR Nisha, KK Aathira and JS Haima

DOI: https://doi.org/10.22271/tpi.2021.v10.i2e.5693

Abstract
The present study was carried out to evaluate the cytotoxic and antiproliferative activity of methanolic extract of *Asparagus racemosus* in MDAB-231 cells. The qualitative phytochemical analysis of the extract revealed the presence of steroids, alkaloids, flavonoids, glycosides, saponins and diterpenes. MDAMB-231 cells were maintained in RPMI media containing 10 per cent serum and 1 per cent antibiotic and antimycotic solution. The cells were harvested and seeded to 96 well plate at 5×10^3 cells/mL, incubated for 24 hours at 37˚C with 5 per cent CO_2. The cells were treated with 160, 80, 40, 20 and 10µg/mL of the extract for 24 hours and viability was accessed using MTT Assay. Also cells were plated in 6 well plates at concentrations 3×10^5 cells/mL and exposed to 80, 40, 20 and 10µg/mL of the extract of *Asparagus racemosus* for acridine orange ethidium bromide (AO/EB) staining. There was a dose dependent decrease in viability of cells exposed to methanolic extracts of *Asparagus racemosus* with a viability of 15.55±0.193 per cent for cells treated with 160µg/mL. The IC_{50} was found to be 91.36± 0.87 µg/mL. The AO/EB staining revealed that most of the cells were dead in extract treated with 80µg/mL where more than 90 per cent of cells were live when treated with 10µg/mL of the extract.

Keywords: *Asparagus racemosus*, oestrogen, phytoestrogens

1. Introduction
The earliest known mention of the utility of medicinal plants dates back to the Vedic times. India being one of the ethno-culturally diversified countries comprising people belonging to different creed and cultures wherein they practice their own herbal remedies for various ailments. India being one of the medico-culturally diverse countries where the use of medicinal plants, is a integral part of traditional medicine systems like Ayurveda, Unani and Siddha. Various lead molecules for the anticancer drugs are being contributed by nature. Analysing the phytoconstituents and conducting their pharmacological and toxicological studies forms the basis of all the lead molecules. The MDAMB-231 cell line is oestrogen receptor (ER) -ve, progesterone receptor (PR) -ve and human epidermal growth factor receptor (HER) –ve and is used as a model for hormone independent breast cancer studies.
Asparagus racemosus (*A. racemosus*) belonging to family Asparagaceae, commonly known as Shatavari, whose tubers are well known for its immunostimulatory, antioxidant, rejuvenating, galactagogue, oestrogen modulatory activities [1]. The rasayana herb is used traditionally in the rejuvenation in female reproductive disorders. *A. racemosus* has been mentioned in Ayurveda for its phytoestrogenic properties and is used in lactation. Thus with this notion we took forward the present study to evaluate the cytotoxic and antiproliferative potential of methanolic extracts of *A. racemosus.*

2. Materials and Methods
2.1 Plant Extraction
The tubers of *A. racemosus* were collected locally, from Mannuthy and was dried in shade until they were completely dry. The tubers were coarsely powdered using an electric pulveriser and the powder obtained was extracted using a Soxhlet extraction apparatus with methanol. The methanol extract was then concentrated using a rotary vacuum evaporator under reduced pressure and temperature (40 ºC). The yield of the extract was calculated and kept under refrigeration in an airtight container after complete evaporation of the solvent until further use.

2.2. Phytochemical analysis
The qualitative phytochemical analysis was performed [2].

2.3 Gas Chromatography and Mass Spectrometry
The active phytochemical principles of MAR and MBD were analysed using GC-MS system of Centre for Analytical Instrumentation- Kerala (CAI-K), Kerala Forest Research Institute (KFRI), Peechi, Kerala. Gas chromatography Mass Spectrometer (Shimadzu GC-MS, Japan, QP2010S) with a mass range of 1.5- 1000 m/z was used. Helium was used as the carrier gas at flow rate of 1 mL/ min. The oven temperature was maintained at 80 ºC for 4 min and then increased to 280 ºC in 6 min. The injector temperature was 260 ºC and total analysis time was 50 min. Aliquots of extracts (0.4 µL) were injected into the chromatographic column after a clear baseline was obtained. Major constituents were identified using mass spectrum library (NIST 11 and WILEY 8).

2.4 Assessment of effect of methanolic extract of *A. racemosus* on viability of MDAMB231 cells and Calculation of IC_{50}
The MTT assay was done using methanolic extracts of *A. racemosus* in MDAMB231 cells at the doses of 160, 80, 40, 20 and 10 µg/mL

Cytotoxic evaluation of methanolic extract of *A. racemosus* was assessed using 3-(4,5- dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay in MDA-MB-231 human breast carcinoma cell lines [3]. The MDA-MB- 231 breast cancer cell line was seeded at a density of 5 x 10^3 cells per well in 200 µL medium and were allowed to attach for overnight in a CO_2 incubator. Cells were treated with methanolic extracts of *A. racemosus* at concentrations of 160, 80, 40, 20 and 10 µg/mL for a period of 24 h. After treatment with plant extracts, the media was removed and 10 µL of MTT (5 mg/mL) in 100 µL medium was added and incubated at 37˚C for 4 hours. Then the media with MTT was removed and the formed purple formazan crystals were dissolved in 200 µL of DMSO and read at 570 nm in an ELISA plate reader (Varioskan Flash, Thermo Fischer Scientific, Finland).

The per cent cell viability and per cent cell inhibition were calculated using the following formulae:

Per cent cell viability = (Average absorbance of treated cells/Average absorbance of untreated cells) × 100

Per cent cell inhibition = 100 - per cent cell viability

The net absorbance from the control wells was taken as 100 per cent viable. The inhibitory concentration 50 (IC_{50}) values of extracts were calculated by plotting the concentration against per cent cell inhibition using online curve fitting software.

2.5. Microscopic studies
2.5.1. Acridine orange/Ethidium bromide Staining
Trypsinized cells at a concentration of 1x 10^5 cells were seeded into a six-well plate and allowed to grow for 24 h. Based on the MTT assay, the IC_{50} concentration of the plant extract were selected for the study. The cells were exposed to concentrations of 80, 40, 20 and 10µg/mL of the extract of *A. racemosus* for 24 hours, the acridine orange-ethidium bromide (AO/EB) staining procedure was followed to differentiate the live, apoptotic and necrotic cells. After discarding media from wells of treated or untreated cells, they were stained with 200 µL of acridine orange (10µg/mL) and ethidium bromide (10µg/mL) and analysed under Trinocular Research fluorescence Microscope (Axio Vert. A1 FL-LED, Carl Zeiss) with blue excitation (488 nm) and emission (550 nm) filters at 20X and 40X magnification [4].

3. Results and Discussion
3.1 Phytochemical Analysis
The results of the phytochemical analysis is summarised in table 1. The qualitative phytochemical analysis revealed the presence of alkaloids, glycosides, tannins, flavonoids, saponins and phenols.

Table 1: Phytochemical constituents of methanolic extract of *A. racemosus*

Phytochemical Test	Methanolic extract of whole plant of *A. racemosus*
Alkaloids	+
Glycosides	+
Tannins	+
Flavonoids	+
Saponins	+
Phenols	+

3.2 Gas chromatography- mass spectroscopy analysis of phytochemicals in methanolic extract of *A. racemosus*

Table 2: Gas chromatography- mass spectroscopy analysis of phytochemicals in methanolic extract of *A. racemosus*

Selected compounds	Retention time	Probability
5-Hydroxymethylfurfural	5.299	14.23
1,2,3-Propanetriol	5.669	2.25
(S)-(-)-1,2,4-Butanetriol, 2-acetate	6.752	0.61
Cycloheptasiloxane, tetradecamethyl-	7.519	6.85
3-furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-	8.549	6.64
2-Nonene	8.728	0.84
Guanosine	9.518	14.86
Cyclooctasiloxane, hexadecamethyl-	10.176	4.02
alpha.-Methyl-l-sorboside	10.612	3.95
delta.1,.alpha.-Cyclohexaneacec acid	11.136	7.53
3,3-dimethyl-5-oxocyclohexanecarbaldehyde	12.118	10.09
Heptasiloxane, hexadecamethyl-	12.192	1.75
Hexadecanoic acid, methyl ester	13.655	3.01
Linoleic acid,methyl ester	15.965	4.25
9-octadecenoic acid (z)-, methyl ester	16.035	5.40
Octadecanoic acid, methyl ester	16.365	1.26

2-Monopalmin	21.954	1.03
Pent-3-ene-2-one, 3-phenyl-, oxime	22.319	0.45
Sigmasterol	23.287	3.85
gamma-Sitosterol	25.906	4.39
Stigmasta-5,22-Dien-3-Ol	34.540	2.76

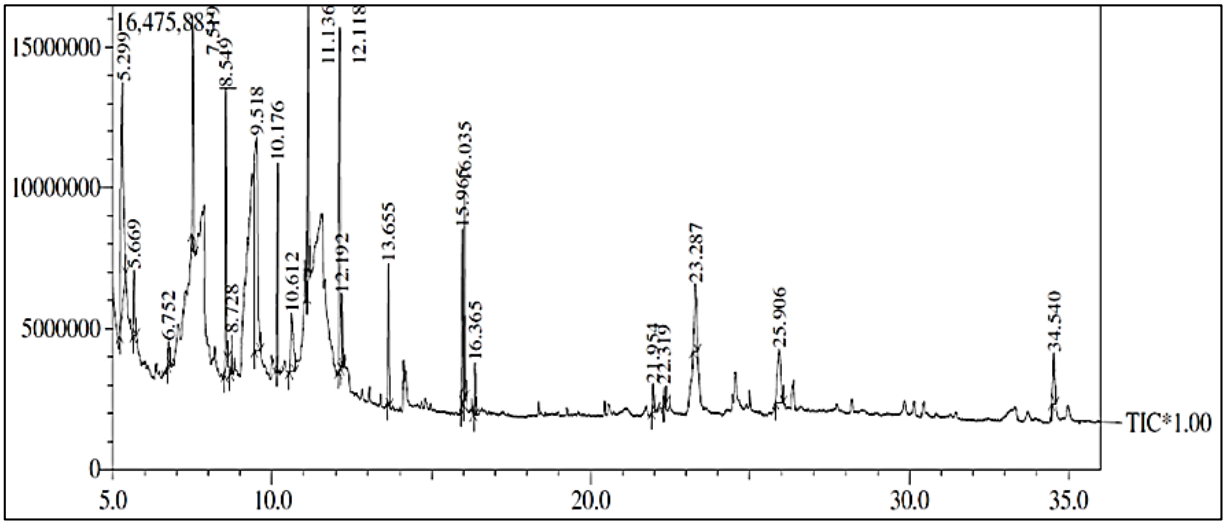

Fig 1: Gas chromatography- mass spectroscopy chromatogram of methanolic extract of *A. racemosus*

3.3 Assessment of effect of extracts on viability of MDAMB 231cells and Calculation of IC_{50}

There was a dose dependent decrease in the viability of cells exposed to different concentrations of extract with the viability being depicted in the figure no.2. The IC_{50} of methanolic extract of *A. racemosus* was 91.36± 0.87 µg/mL as obtained from MTT assay.

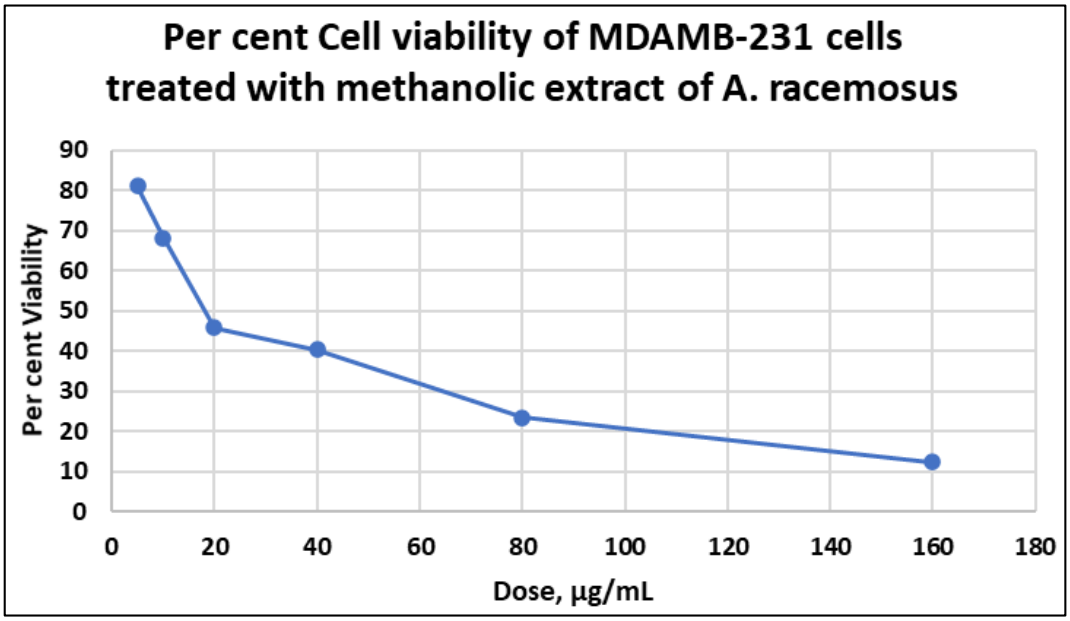

Fig 2: Graph depicting the Per cent Cell viability of MDAMB-231 cells treated with methanolic extract of *A. racemosus*

3.4. Microscopic studies

3.4.1. Acridine orange/Ethidium bromide (AO/EB) staining

The results of apoptotic stages analysed by AO/EB staining upon treatment of cells with methanolic extract of *A. racemosus* are depicted in the figure no.3. Control cells emitted uniform green fluorescence with circular nucleus in the centre whereas methanolic extract of *A. racemosus* treated cells showed early apoptosis featured by yellow-green fluorescence and crescent shaped nucleus. Cellular changes such as condensation and fragmentation of nucleus, nuclear marginalisation and formation of apoptotic bodies were noticed in cells treated with the extract. Majority of the cells were dead when treated with extract 80 µg/mL.

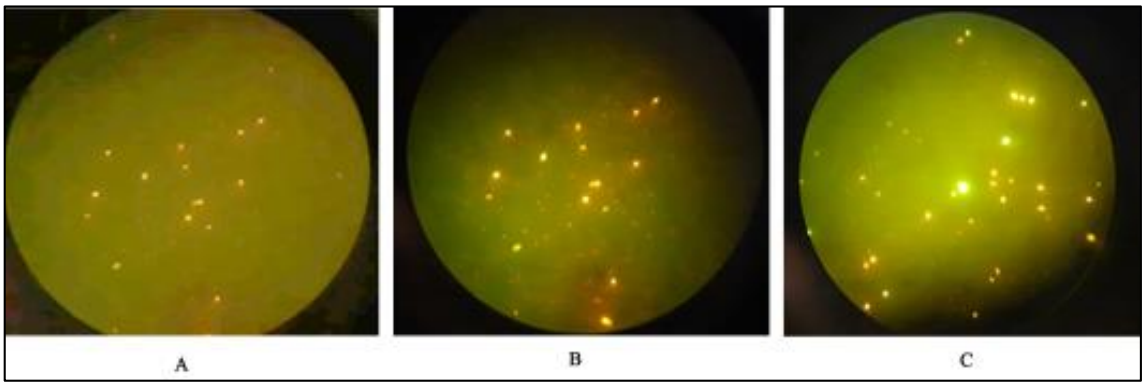

Fig 3: Showing cells treated with methanolic extracts of *A. racemosus* after AO/EB staining
A- represents cells treated with 80microgam/mL, containing orange-coloured cells showing stages of apoptosis B and C contain both orange and yellow fluoroscence, treated with 40 and 20 micrograms/mL of extract

4. Discussion

Effect of various phytochemicals on the live cells and their cytotoxicity can be assessed using various assays including the MTT assay and LDH Assay. In MTT assay, live cells convert the chemical MTT to purple formazan, the colour of which is read spectrophotometrically [5]. In the present study, there was a dose dependent decrease in the viability of cells exposed to methanolic extract of *A. racemosus.* The decrease in viability indicated that the extract has potent cytotoxic action. There was decrease in viability of cells exposed to methanolic extracts of *A. racemosus* with lowest viability being 15.55±0.193 per cent for cells treated with 160µg/mL. Previous studies indicated that 5-hydroxy methyl furfural [6] can cause antiproliferative action against cells and the presence of the phytochemical, as per GC-MS, can be the major reason for the cytotoxicity.

The cells undergoing apoptosis can be differentiated on the basis of their morphological characteristics such as blebbing of the plasma membrane with irregular outlines, chromatin condensation and cytoplasm shrinkage with the apoptotic bodies [7]. The usage of fluorescence light microscopy with differential uptake of DNA binding fluorescent dyes such as ethidium bromide and acridine orange, as the most preferred method for apoptosis detection [8]. In the present study, the cells that were treated with the higher concentrations of the extract showed signs of apoptosis as indicated by the orange fluroscence and the cells treated with the lowest concentrations were more or less alive. The AO/EB staining revealed that most of the cells were dead in extract treated with 80µg/mL where more than 90 per cent of cells were live when treated with 10µg/mL of the extract. Further characterization and documentation of the compound needs to be performed isolate the phytochemical responsible for the antiproliferative activity of *A. racemosus.*

5. Acknowledgements

The authors are thankful to the College of Veterinary and Animal Sciences, Mannuthy, under Kerala Veterinary and Animal Sciences University for providing the facilities financial assistance provided.

6. References

1. Bopana N, Saxena S. *Asparagus racemosus*-Ethnopharmacological evaluation and conservation needs. Journal of ethnopharmacology 2007;110(1):1-15.
2. Harborne AJ. Phytochemical Methods a Guide to Modern Techniques of Plant Analysis. (3rd Ed.). Chapman and Hall, London, UK 1998, 295p.
3. Riss TL, Moravec RA. Use of multiple assay endpoints to investigate the effects of incubation time, dose of toxin, and plating density in cell-based cytotoxicity assays. Assay and drug development technologies 2004;*2*(1):51-62.
4. Ribble D, Goldstein NB, Norris DA, Shellman YGA. simple technique for quantifying apoptosis in 96-well plates. BMC biotechnology 2005;5(1):1-7.
5. Bauer D, Redmon N, Mazzio E, Soliman KF. Apigenin inhibits TNFα/IL-1α-induced CCL2 release through IKBK-epsilon signaling in MDA-MB-231 human breast cancer cells PLoS One 2017 [online]
6. Zhao L, Chen J, Su J, Li L, Hu S, Li B, *et al. In vitro* antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. Journal of agricultural and food chemistry 2013;61(44):10604-10611.
7. Searle J, Kerr JF, Bishop CJ. Necrosis and apoptosis distinct modes of cell death with fundamentally different significance. Pathology Annals 1982;17(6):229-259
8. Renvoize C, Biola A, Pallardy M, Breard J. Apoptosis: identification of dying cells. Cell biology and toxicology 1998;14(2):111-120.