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Original Article

ANTICANCER POTENTIAL OF SALACIA CHINENSIS L. AND WOODFORDIA *FRUTICOSA* (L.) KURZ OF THE WESTERN GHATS FORESTS OF INDIA

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ABSTRACT

Objective: *Salacia chinensis* and *Woodfordia fruticosa* are medicinal plants from the Western Ghats of India traditionally used in the treatment of diabetes, diarrhea and worm infections. The current study aims to evaluate the cytotoxic potential of methanolic extract of *Salacia chinensis* and *Woodfordia fruticosa* against breast and pancreatic cancers.

Methods: Methanolic extract of dried leaves of *Salacia chinensis* and *Woodfordia fruticosa* were obtained by Soxhlet extraction. The cytotoxic potential of the dried extract was evaluated against human breast (MDA-MB-231) and pancreatic (PANC-1) cancers *in vitro* using MTT-based assay.

Results: The study showed a dose-dependent cytotoxicity of *Salacia chinensis* and *Woodfordia fruticosa* leaf extracts against breast and pancreatic cancers with IC_{50} values of 124 µg/ml against MDA-MB-231 and 230.5 µg/ml against PANC–1 cells, respectively.

Conclusion: Results indicate the presence of cytotoxic phytochemicals in *Salacia chinensis* and *Woodfordia fruticosa.* Further purification of the extract might be beneficial to isolate the anticancer phytochemical.

Keywords: *Salacia chinensis, Woodfordia fruticosa,* Breast cancer, Pancreatic cancer, Phytochemical, Cytotoxicity

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INTRODUCTION

Cancer is the second leading cause of death worldwide after cardiovascular diseases [1]. According to GLOBOCON 2020, breast, lung and colorectal cancers are the most commonly seen cancers and pancreatic cancer is one of the rare cancers worldwide [2]. Currently, surgical resection, chemotherapy and radiation therapy are the commonly available treatment options to cancer patients [3]. However, the side effects associated with chemotherapy drugs demand the discovery of newer anticancer drugs [4]. The development of drug resistance, cytotoxicity to normal cells and metastasis of cancer are the major side effects of chemotherapeutic drugs that lead the patient to death [5-9]. The ideal anticancer agent should be able to selectively target the cancerous cells without harming the normal cells [10-12]. Currently, there are several anticancer drugs isolated from plants such as paclitaxel from *Taxus brevifolia* L., vincristine, vinblastine, and vinorelbine from *Catharanthus roseus* G. Don that are used to treat cancer patients [11]. In addition, about 16 plant-derived compounds (eg. *flavopiridol* from the Indian tree *Dysoxylum binectariferum*, *meisoindigo*, from the Chinese plant *Indigofera tinctoria*) are being tested in clinical trials, 13 are in phase I or II and three are in phase III trials [11]. Besides, several polyphenols from different plants are also reported to exhibit antioxidants, antimicrobial, and anticarcinogenic properties [13-16]. The use of *in vitro* and *in vivo* experiments is necessary to screen the bioactive potential of phytochemicals from different sources [12, 15]. However, hardly a limited number of plant resources have been pharmacologically screened for the bioactive potential [17].

In this research, we have used two plants viz, *Salacia chinensis* L. of the family Celastraceae and *Woodfordia fruticosa* (L.) Kurz belongs to the family Lytheraceae, both of which have exhibited several health benefits and curative properties with respect to health conditions like type 2 diabetes, mutagenicity, hepatitis, cardiac disorders, mental disorders and insulin resistance; with documented antimicrobial, antioxidant, immunomodulatory, anti-infertility and antitumor activities in several *in vitro* studies [18-20]. The water extract of *Salacia chinensis* L. (SC) stem or 'Kumpang jed chan' in Thai has been used as a folk remedy to treat patients with cirrhosis in a local hospital with promising results [18]. Different parts of this plant contain many biologically active compounds, such as triterpenes, phenolic compounds, flavonoids. The solvent extracts of *S. chinensis* root and stem showed potent antioxidant, antiulcer, antidiabetic, hypoglycemic, antiobesity and skin-lightening properties [18]. Further, the cytotoxic effect of *S. chinensis* was reported against lung (LU), epidermal (KB), liver (Hep-G2) and breast (MCF-7) cells were also reported [21]. A wide range of pharmacological properties, including antihyperglycemic, antioxidant, anti-inflammatory, analgesic, hepatoprotective, antibacterial, gastroprotective and wound healing properties of *Woodfordia fruticosa* have been recorded in a recent review by Giri *et al.* [20]. *In vitro* cytotoxic potential of methanolic extract of *W. fruticosa* flowers was reported against liver cancer (PLC/PRF/5) and brine shrimp larvae were reported [22, 23]. To the best of our knowledge, the cytotoxic effect of methanolic extract of *S. chinensis* and *W. fruticosa* were not studied against breast cancer–MCF7 cells and pancreatic cancer-PANC-1 cells and, therefore, we have evaluated these parameters in the current study using MTT based colorimetric analysis.

MATERIALS AND METHODS

Chemicals and consumables

All the chemicals and plastic wares used in the experiment were of cell culture grade purchased from Himedia, Mumbai, India and Tarsons, India. Standard Cisplatin was purchased from a medical shop. The solvents used were of analytical grade and procured from Merck, Mumbai, India.

Plant samples and extraction of phytochemicals

S. chinensis and *W. fruticosa* leaf, along with the young stem samples, were collected from the Arboretum (Latitude: 12° 48' 34.02" N

Longitude: 74° 55' 15.99" E) of Mangalore University Campus, Mangalore, India. The plants were identified using Flora of Presidency of Madras [24] and the voucher specimens (MU/AB/NKC/01 and MU/AB/NKC/02 for *S. chinensis* and *W. fruticosa*) were deposited at the herbarium of the Applied Botany Department, Mangalore University, Karnataka, India. The leaf samples were air-dried under shade for about a week and, extracted in 100% and stored until further use.

The phytochemicals in the dried samples were extracted in methanol using Soxhlet for about 16 h. The extract was collected, filtered and concentrated to dryness using a vacuum concentrator at 45 °C. The dried extract was stored under refrigerated conditions until use.

Cell lines and cell culture

Human breast cancer epithelial cell line-MDA-MB-231 and human pancreatic cancer cell line-PANC–1 was purchased from the National Centre for Cell Science (NCCS), Pune, India and grown in Dulbecco's modified Eagle's medium-DMEM (Himedia, Mumbai, India) supplemented with 10% foetal bovine serum (Himedia, Mumbai, India) and 1% penicillin/streptomycin (Himedia, Mumbai, India), and incubated under 5% CO_2 incubator at 37 °C.

Preparation of test sample to evaluate the cell viability

The methanol extract of *S. chinensis* and *W. fruticosa* (1 mg/ml) was dissolved in DMSO and made up to the final volume using DMEM medium. The concentration of DMSO was maintained at less than 0.1% while preparing the sample. The samples were filtered using 0.22 µm syringe filters.

Cell viability assay

Cell viability was determined by MTT-(3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide)-based assay [25]. A hundred microliters of MDA-MB-231 and PANC-1 cells at a cell density of 1x105cells/ml were seeded in a 96-well microtiter plate and incubated overnight under a 5% CO2 incubator at 37°C. The cells were treated with or without S. chinensis and W. fruticosa samples at varying concentrations of 12.5-200 µg/ml for 48 h. Standard drug Cisplatin was used at a concentration of 3.12-50 µg/ml. Cell viability was assessed after the addition of MTT and recording the absorbance at 570 nm using a microplate reader (Synergy H1, BioTek Instruments Inc., USA).

Statistical analysis

All the experiments were tested in triplicate and the data was expressed as mean±standard deviation (SD). Statistical analysis of the data was performed by one-way ANOVA using SPSS 21 software at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

A significant ($p<0.05$) dose-dependent cytotoxicity was observed for the standard drug cisplatin and the extracts of both *S. chinensis* and *W. fruticosa* against breast and pancreatic cancers (table 1; fig. 1-3). The IC_{50} value for cisplatin was 2.54 µg/ml and 7.232 µg/ml against MDA-MB-231 and PANC-1 cells, respectively. The crude extract of *S. chinensis* showed IC_{50} values of 124 µg/ml and 230.5 µg/ml against MDA-MB-231 and PANC–1 cells, respectively, while *W. fruticosa* showed an IC_{50} value of 126.53 µg/ml and 91.15 µg/ml against MDA-MB-231 and PANC 1 cells respectively (table 2).

Table 1: Cytotoxic effect of *S. chinensis* and *W. fruticosa* against MDA-MB-231 and PANC-1 cells

Sample	Concentration (µg/ml)	% Cytotoxicity (mean±SD)	
		MDA-MB-231	PANC-1
Cisplatin	0	0	0
	3.12	50.58±4.30	38.63±4.83
	6.25	54.54±2.02	51.12±1.28
	12	61.75±5.44	59.34±3.72
	25	71.83±2.02	73.96±0.25
	50	90.83±5.81	9.37±1.24
S. chinensis	0	0	0
	12.5	40.05±3.69	27.59±1.03
	25	40.23±5.40	36.88±1.91
	50	46.97±4.36	34.52±0.73
	100	49.09±3.22	39.21±2.48
	200	66.77±4.36	46.30±2.21
W. fruticosa	0	0	0
	12.5	14.40±3.11	22.32±2.62
	25	44.38±5.37	29.79±4.77
	50	44.95±5.29	39.91±7.18
	100	50.92±1.51	51.49±4.07
	200	53.09±7.19	58.14±1.27

The mean±SD values are obtained by taking the average and standard deviations of the results from 3 trials for each of the experiments with n=3 wells.

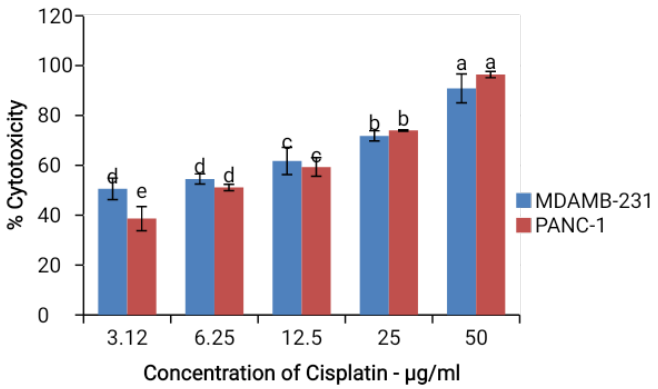

Fig. 1: Effect of Cisplatin on MDA-MB-231 and PANC–1 cells. All the values are obtained by taking the average of the results from 3 trials for each of the experiments with n=3 wells and standard deviations are expressed as error bars. Means with different alphabets (a-d) represent significant differences at 5% level

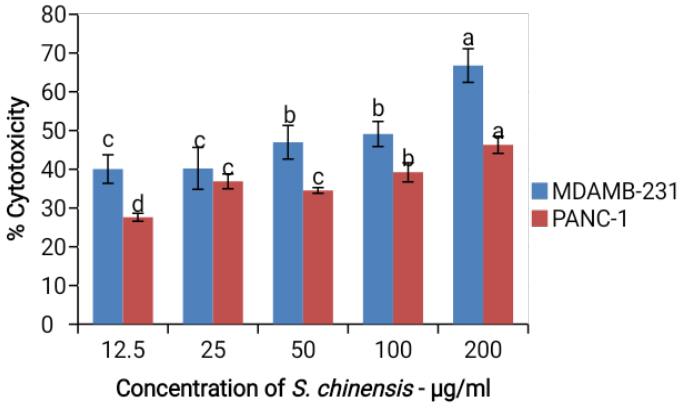

Fig. 2: Effect of *S. chinensis* on MDA-MB-231 and PANC-1. All the values are obtained by taking the average of the results from 3 trials for each of the experiments with n=3 wells and standard deviations are expressed as error bars. Means with different alphabets (a-d) represent significant differences at 5% level

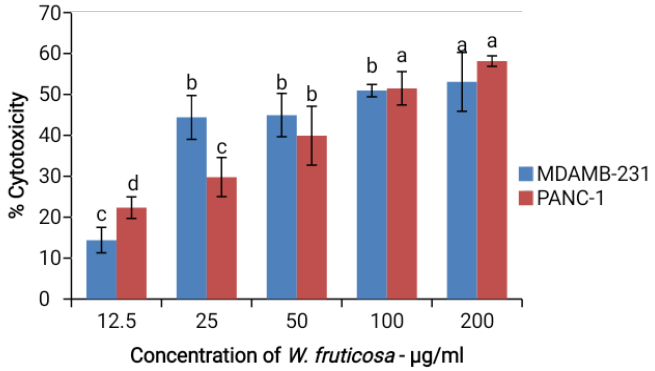

Fig. 3: Effect of *W. fruticosa* on MDA-MB-231 and PANC-1. All the values are obtained by taking the average of the results from 3 trials for each of the experiments with n=3 wells and standard deviations are expressed as error bars. Means with different alphabets (a-d) represent significant differences at 5% level

Table 2: IC_{50} values of *S. chinensis, W. fruticosa* and cisplatin on MDA-MB-231 and PANC-1 cells

Sample	IC_{50} values* (µg/ml)	
	MDA-MB-231	PANC-1
Cisplatin	2.54	7.232
S. chinensis	124	230.5
W. fruticosa	126.53	91.15

*All the values are obtained by taking the average of the results from 3 trials for each of the experiments with n=3 wells.

In a study carried out by Khalid *et al.* [25], the anticancer activity of *Sisymbrium officinale* plant extract was noticed at 100 µl of the plant extract on the MCF7 (breast cancer cells) with 6% cancer cell death [26]. However, no results were shown on the 50 µl, 200 µl, or 400 µl concentrations. Musini *et al.* observed the concentration-dependent antiproliferation activity of the methanol extract of *Salacia oblonga* on the breast cancer cell lines (MDA-MB-231) [27]. The results indicated that after treatment with plant extract at a concentration of 30 µg/ml, the cell viability decreased dramatically. Based on their observations, the $IC_{50,}$ value for methanolic aerial and root extracts on breast cancer cells was 35 and 44 µg/ml, respectively after 24 h of incubation. One of the fractions eluted by methanolic extract of *S. oblonga* also gave a positive cytotoxic effect on EAC with 75% cell death at a concentration of 25 µg/ml and 100% at 50 µg/ml [28].

Ethanolic extract of *W. fruticosa* flowers has been shown to possess anticancer properties in the human liver's PLC/PRF/5 cell lines [22]. On the basis of MTT assay, it was concluded that the synergistic effect of the phytochemicals present in the extract might be responsible for the potential chemoprevention property of *W. fruticosa* flowers in hepatic cancer [22].

Even in the present study, the cytotoxicity of the extracts of both *S. chinensis* and *W. fruticosa* increased with increasing concentrations up to 200 µg. The study revealed promising antiproliferative effect of *W. fruticosa* against PANC–1 cell lines, demonstrating an IC_{50} value of 91.15 µg/ml, while both *W. fruticosa* and *S. chinensis* demonstrated moderate antiproliferative effect against MDA-MB-231 cell lines. Probably, the phytochemicals in the extract might be involved in regulating the molecular pathways that are implicated in the growth and progression of cancer, as mentioned by Choudhari *et al.* [3]. MDA-MB-231 is a triple-negative breast cancer and phytochemicals in the crude extract of *W. fruticosa* and *S. chinensis* are less active compared to PANC-1 cells. Though our study suggests the possible anticancer potential of *W. fruticosa* and *S. chinensis*, further experiments on the isolation of individual phytochemicals and evaluation of their cytotoxicity against breast and pancreatic cancers are necessary to take it further to drug development.

CONCLUSION

Salacia chinensis and *Woodfordia fruticosa* are medicinal plants from the Western Ghats of India traditionally used in the treatment of

diabetes, diarrhea and worm infections. The current study aims to evaluate the cytotoxic potential of methanolic extract of *S. chinensis* and *W. fruticosa* against breast and pancreatic cancers *in vitro*. The study revealed a dose-dependent cytotoxicity of both *S. chinensis* and *W. fruticosa* against both MDA-MB-231 and PANC-1 cells. The results were compared with the standard cisplatin. Among the 2 plants used, *W. fruticosa* extract showed a lower IC_{50} value of 91.15 µg/ml against pancreatic cancer cells compared to breast cancer. Also, *S. chinensis* showed a higher IC_{50} value. Probably, the phytochemicals present in the extracts are more active against pancreatic cancer compared to breast cancer cells. However, further experiments on the isolation, characterization, and validation of the phytochemical and its cytotoxicity are necessary for further use of this plant in the pharmaceutical industry to develop an anticancer drug.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

All the authors have none to declare

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