Saudi Journal of Medical and Pharmaceutical Sciences **ISSN 2413-4929 (Print)**
Scholars Middle East Publishers **ISSN 2413-4910 (Online)**
Dubai, United Arab Emirates
Website: https://saudijournals.com/ DOI: 10.36348/sjmps.2017.v03i05.001

Original Research Article

Novel Prediction of Anticancer Drug Screening in Cancer Cell Lines by SRB Assay

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Abstract: Medicinal plants contain numerous bioactive phytochemicals or bionutrients. Various studies carried out during the past 2– 3 decades on these phytochemicals reveal their important role in preventing chronic diseases like cancer, diabetes and coronary heart disease. The major classes of phytochemicals with disease-preventing functions are dietary fibre, antioxidants, anticancer, detoxifying agents, immunity-potentiating agents and neuropharmacological agents. Each class of these useful agents consists of a wide range of chemicals with immense potential. Some of these have more than one function. In the present work the sulphorhodamine B (SRB) assays of *Shorea robusta* oleoresin, the triterpenes amyrenol isolated from its defatted portion and W*rightia tinctoria* bark ethanol extract were carried out. Amyrenol showed the best highest activity and lowest IC_{50} value (37.56, 11.61 and 61.14 µg/mL) with cervical cell lines while the other extracts also registered fairly good activity. Amyrenol merits further evaluation as an anticancer agent.

Keywords: incompatible, dietary fibre, phytochemicals, neuropharmacological agents, sulphorhodamine B, defatted fraction

INTRODUCTION

There is a compelling need to explore new alternative and complementary medicine with anticanceractivity. It has been observed that ethnomedicinal plants frequently serve as sources of new drugs with little or no side effects [1]. There is, much scope for systematic research in screening Indian medicinal plants for these phytochemicals and assessing their potential in protecting against different types of diseases [2]. Cytotoxicity screening assay is one of the most important methods to assess the survival of cell. Two major techniques are used to assess the cell growth. The first one uses either 3-(4, 5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) or 2,3-bis(2-meth- oxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide sodium salt (XTT).

The second major technique for testing cytotoxicity is the more preferred sulphorhodamine B (SRB) assay. SRB assay is used for cell density determination based on the measurement of cellular protein content. This relies on the uptake of the negatively charged pink aminoxanthine dye, SRB by basic amino acids in the cells. Greater the number of cells, larger will be the amount of dye taken up .After fixing, when the cells are lysed, the released dye gives more intense colour and greater absorbance. It is sensitive, simple, reproducible and more rapid than the formazan based assays and gives better linearity, a good signal-to-noise ratio and has a stable end-point that does not require a time-sensitive measurement [3]. The cell lines used are SKMEL-28, HELA and HCT-15 skin carcinoma, cervical cancer and colon cancer.

MATERIALS AND METHODS

S. robusta oleoresin and *W. tinctoria* barks were collected from Idukki District, Kerala, India. These were authenticated by Mr. Rogimon. P. Thomas, Assistant Professor Department of Botany, C.M.S College, Kottayam, Kerala, India.

Shade dried plant materials of *Shorea robusta* oleoresin and *Wrightia tinctoria* bark were soaked in 95% ethanol overnight and then refluxed for three hours; the clear extract was decanted off, the procedure being repeated thrice [4]. The extracts were pooled and concentrated by distillation under reduced pressure till a syrupy consistency was achieved. Solvent was

evaporated to dryness on a water bath. Total ethanol extracts (TEE) of both the plants were thus prepared and the yield recorded.

A known triterpene amyrenol was isolated from the defatted fraction of *S. robusta* oleoresin by column chromatography. The dry TEE of *S. robusta* oleoresin and *W. tinctoria* barks were used for the SRB assay.

***In vitro* cytotoxicity by SRB assay**

The assay is based on the uptake of the negatively charged pink aminoxanthine dye, sulphorhodamine B (SRB) by basic amino acids in the cells [5]. The greater the number of cells, the greater amount of dye is taken up and after fixing, when the cells are lysed, the released dye will give a more intense colour and greater sensitivity [6].

All the cell lines were purchased from NCCS Pune and was maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°c in 5 % CO2 (NBS, EPPENDORF, GERMANY) in a humidified atmosphere in a CO2 incubator. The cells were trypsinized (500µl of 0.025% trypsin in PBS/ 0.5mM EDTA solution (Himedia)) for 2 minutes and transferred to T flasks in complete aseptic 96 well plate, on which the cells were previously grown, were used to add the sample with a varying concentration of 10, 25, 50,75 and 100 µg/ml. The OD was read in a microplate reader at 510nm [7].

Cytotoxicity testing is based on one or more mammalian cell lines grown under surroundings where they actively grow and undergo mitotic division [8]. A diversity of experiments has been used and the most basic is to compare the rate of proliferation of a cancer cell line in presence and absence of the test substance, usually after a specified time.

RESULTS AND DISCUSSION

SRB assay is a high-throughput and sensitive method for evaluating cytotoxic activity against cancer and non- cancerous cell lines. It has a number of advantages over other current cytotoxicity assays; because SRB assay is independent of cell metabolic activity, not interfered by test compounds and easy to perform [1].

The presence of medicinally significant molecules in a plant is indicative of its medicinal potential. Phytochemical screening of the plants *S. robusta* oleoresin and *W. tinctoria* barks confirmed the presence of indole, alkaloids, flavones, triterpenoids and fatty acids in the plants [9, 10]. The SRB assay of the plants and the isolated compound amyrenol were carried out using SKMEL-28, skin cancer cell lines, HELA, cervical cell lines and HCT-15, colon cancer cell lines.

Table 1: SRB Assay of on SKMEL-28 Cell lines showing optical density, % viability & IC_{50} Values.

Sl.No.	Sample	Sample concentration (µg/mL)	Optical density(Mean ± SEM)	% viability(Mean ± SEM)	IC_{50} (µg/mL)
1	***Shorea robusta* (SR)**	10	0.1813 ± 0.005	67.96 ± 0.947	154.30
		25	0.1668 ± 0.001	65.30 ± 0.2210	
		50	0.1606 ± 0.001	60.44 ± 0.4626	
		75	0.1504 ± 0.0003	59.24 ± 0.1201	
		100	0.1431 ± 0.0009	57.27 ± 0.3622	
2	***Wrightia tinctoria* (WT)**	10	0.2141 ± 0.007	59.58 ± 1.1939	48.37
		25	0.1987 ± 0.0006	54.81 ± 0.342	
		50	0.1839 ± 0.001	52.77 ± 0.608	
		75	0.1803 ± 0.0003	49.43 ± 0.104	
		100	0.1743±0.001	47.03 ± 0.313	
3	**Amyrenol (SR-1)**	10	0.1632± 0.002	53.63 ± 0.711	37.56
		25	0.1549 ± 0.008	50.9 ± 0.2829	
		50	0.1469 ± 0.001	48.04 ± 0.2571	
		75	0..1410 ± 0.001	46.35 ± 0.1707	
		100	0.1312 ± 0.003	43.13 ± 1.0314	

Values are expressed as Mean ± SD, n=3

SKMEL-28 cell lines given good observation for total ethanolic extract of *W. tinctoria* bark when compared to total ethanolic extract *S. robusta* oleo resin.

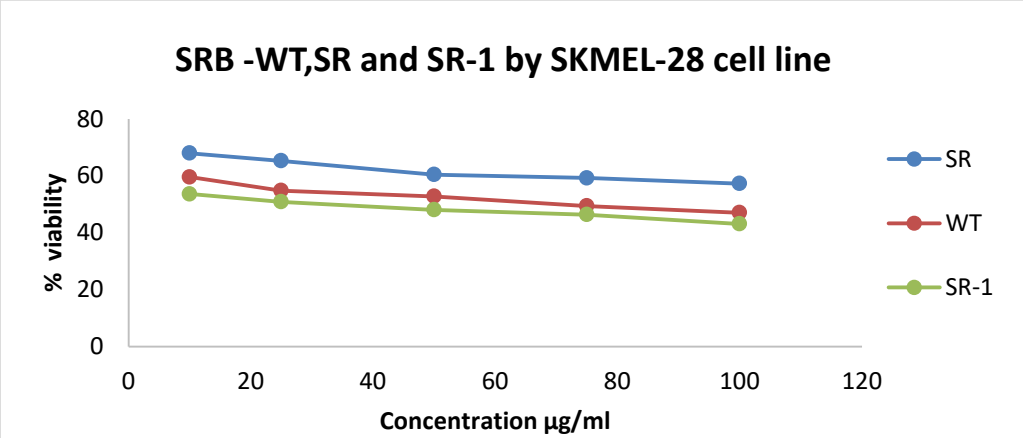

Fig-1: Comparative evaluation of IC_{50} values of *S. robusta, W. tinctoria* and amyrenol on SKMEL-28 cell line by SRB assay

The isolated compound amyrenol showed good activity when compared with that of the other two extracts.

Table 2: SRB Assay of on HeLa cell lines showing optical density, % viability & IC_{50} values

Sl. No	Sample	Sample concentration(µg/mL)	Optical density (Mean ± SEM)	% viability (Mean ± SEM)	IC_{50}(µg/mL)
1	***Shorea robusta* (SR)**	10	0.3737± 0.008	86.61 ± 0.6695	82.39
		25	0.3094 ± 0.002	70.59 ± 0.5863	
		50	0.2609 ± 0.004	59.52 ±1.075	
		75	0.2322 ± 0.001	52.97 ± 0.375	
		100	0.198 ± 0.0004	45.17 ± 0.094	
2	***Wrightia tinctoria* (WT)**	10	0.4078± 0.006	93.04 ± 1.394	92.22
		25	0.3488 ± 0.003	79.58 ± 0.7721	
		50	0.2799 ± 0.007	63.86 ± 1.764	
		75	0.2577 ± 0.002	58.81 ± 0.5407	
		100	0.2136 ± 0.002	48.74 ± 0.5590	
3	**Amyrenol (SR-1)**	10	0.2193± 0.0004	50.04 ± 0.1072	11.61
		25	0.2054± 0.002	46.86± 0.5303	
		50	0.1939± 0.001	44.24± 0.4297	
		75	0.1542± 0.001	35.19± 0.2946	
		100	0.1424± 0.0001	32.48 ± 0.0395	

Values are expressed as Mean ± SD, n=3

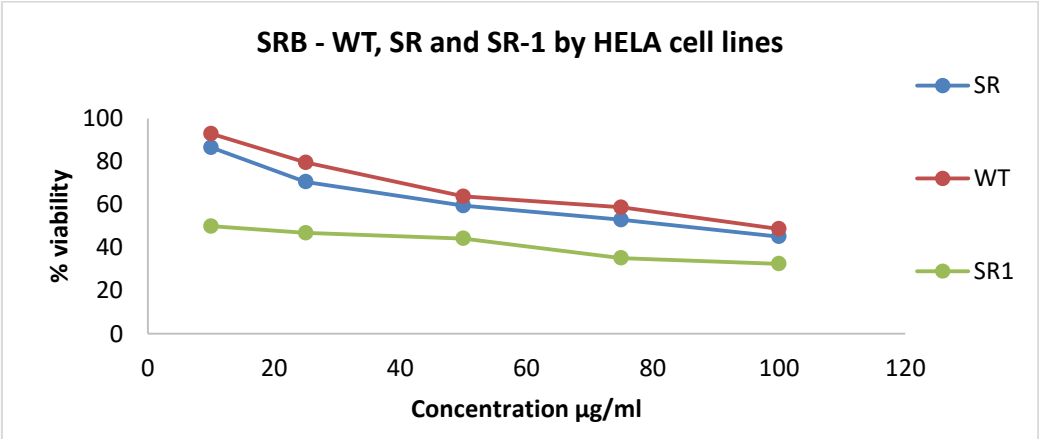

Fig-2: Comparative evaluation of IC_{50} values of *S. robusta, W. tinctoria* andamyrenol on HeLa cell line by SRB assay

The IC_{50} value of TEE *S. robusta* oleoresin showed good activity that of TEE of *W. tinctoria* bark in HeLa cell lines.

Table 3: SRB Assay of on HCT-15 Cell lines showing optical density, % viability & IC_{50} Values

Sl.No	Sample	Sample concentration(µg/mL)	Optical density (Mean ± SEM)	% viability (Mean ± SEM)	IC_{50}(µg/mL)
1	***Shorea robusta* (SR)**	10	0.4081± 0.012	58.11 ± 2.5143	72.64
		25	0.3597± 0.003	65.11± 0.5303	
		50	0.2863± 0.005	51.81± 0.4297	
		75	0.2592± 0.0007	46.92± 0.2946	
		100	0.2506± 0.004	45.35 ± 0.0395	
2	***Wrightia tinctoria* (WT)**	10	0.4202± 0.013	76.06± 2.5143	75.52
		25	0.3287± 0.001	59.30± 0.1870	
		50	0.2817± 0.004	50.98± 0.8735	
		75	0.2759± 0.002	49.93± 0.4526	
		100	0.2587± 0.004	46.82± 0.7625	
3	**Amyrenol (SR-1)**	10	0.4135± 0.003	74.84± 2.5143	61.14
		25	0.3678± 0.004	66.58± 0.8037	
		50	0.3236± 0.004	58.58 ± 0.8223	
		75	0.2509± 0.001	45.42 ± 0.3081	
		100	0.1526± 0.0002	27.63 ± 0.0493	

Values are expressed as Mean ± SD, n=3

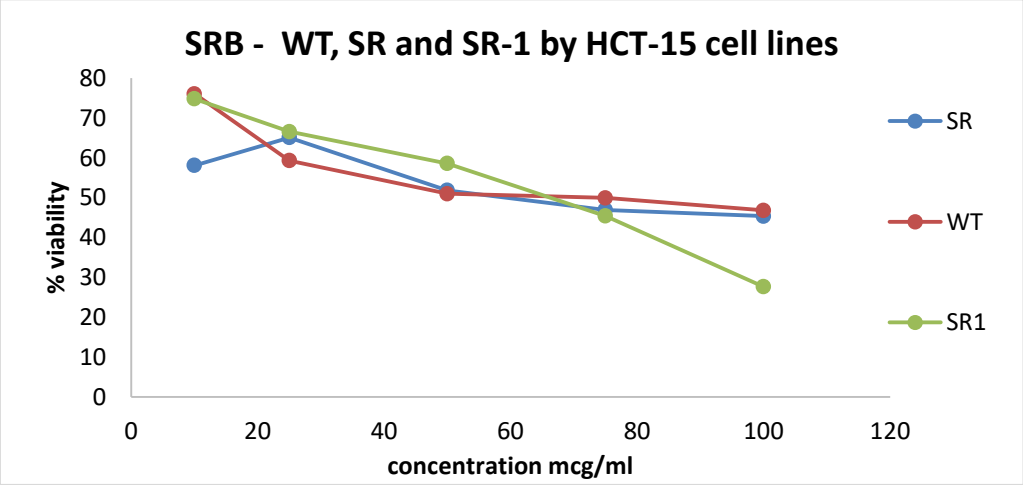

Fig-3: Comparative evaluation of IC_{50} values of *S. robusta, W. tinctoria* and amyrenol on HCT-15 cell line by SRB assay

All the results obtained showed low IC_{50} value for the isolated compound, amyrenol of 37.56, 11.61 and 61.14µg/mL with highest activity. Out of the TEE of selected plants, *W. tinctoria* gave better activity compared to *S. robusta*. An excellent cytotoxicity result was obtained against cervical cell lines.

The results clearly established the supremacy of amyrenol, as far as SRB assay is concerned, using three different cell lines namely SKMEL-28, HCT-15 and HeLa cell lines. The other crude extracts also registered fairly good activity, but less than that of amyrenol. The latter merits further evaluation for its anticancer potential.

CONCLUSION

The total ethanol extract of *W. tinctoria* bark and *S. robusta* oleoresin and the isolated compound, amyrenol from *Shorea robusta*oleoresin were subjected to SRB assay using SKMEL-15, HeLa and HCT-15 cell lines. Among all these amyrenol registered best activity (IC_{50}: 37.56, 11.61 and 61.14 µg/mL) while the other two extracts also gave encouraging results(IC_{50}:154.30, 82.39 and 72.64 µg/mL (*S. robusta*) 48.37, 92.22 and 75.52µg/mL (*W. tinctoria*) in the order of cells given above). Amyrenol registered best activity and lowest IC_{50} value with cervical cell lines. The overall results indicate promising baseline information of the potential uses of amyrenol as an anticancer agent in cervical

cancer which merits evaluation by *in vivo* investigations.

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