International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 15-12-2021, Accepted: 30-12-2021, Published: 15-01-2022
Volume 7, Issue 1, 2022, Page No. 309-312

In vitro anticancer activity of *Sida schimperiana* ethanol extract of callus against MCF-7 human breast cancer cell line

Prabhu V[1], Ramar K[2], Dhinesh V[3], Karthick Kumar A[4], Priyanka B[4]

[1] Assistant Professor, Department of Botany, Selvamm Arts and Science College, Namakkal, Tamil Nadu, India
[2] Assistant Professor, Department of Botany, National College, Trichy, Tamil Nadu, India
[3] Assistant Professor, Soil Science and Agricultural Chemistry, Kumaraguru Institute of Agriculture, Erode, Tamil Nadu, India
[4] Assistant Professor Department of Biotechnology Selvamm Arts and Science College, Namakkal, Tamil Nadu, India

Abstract
We used medicinal plants for more than thousands of years to treat various diseases. They've always been a reliable supply of biologically active medications. WHO report states that nearly 80% of the population, especially in developing countries, rely on a traditional medical approach for healthy sustenance. The anticancer efficacy of various doses of ethanolic extract of Sida schimperiana was investigated experimentally using an in vitro approach. The results showed that different concentrations of the extracts have substantial anticancer efficacy. MTT assay was used to investigate the anticancer activity of an ethanolic extract of *Sida schimperiana* against the MDA MB 231 breast cancer cell line. The sample's IC50 value against breast cancer MDA MB 231 cell lines was 82.50g/ml. We observed significant findings, indicating that this herb can be used in traditional medicine.

Keywords: *Sida schimperiana*, MTT assay, Breast cancer, cell line MDA MB 231

Introduction

Plants have played an essential part in human life for a long time, and their usage as therapies is still prevalent (Kultur 2007) [1]. Herbal treatments are generally well tolerated by women who are battling breast cancer. According to Roberts (2010) and Wong *et al*. (2010), up to 80% of women with breast cancer use complementary or alternative medicine, the most common of which is the use of herbs, in the hopes of reducing the side effects of therapy, enhancing the quality of life, supplying a tremendous control, and relieving stress. Cancer is the world's second-largest cause of death. Breast cancer is the cause of cancer-related mortality in women, accounting for 30% of all cancers in women (Greenlee *et al*., 2000) [2]. (Altobelli and Lattanzi 2014) [5] Breast cancer is a major worldwide health issue and one of the leading causes of death in women. According to Enguita and Fortes (2014), hepatocellular carcinoma is the sixth most prevalent cancer and the third largest cause of cancer-related death [6]. Natural products have long been a valuable source of cancer therapy. Scientists discovered thousands of plants possessing powerful anti-cancer effects (Qi *et al*., 2005). Over 60% of currently used anticancer drugs (Cragg *et al*., 1997) [4] come from natural sources. Schimper's Sida is a woody perennial herb with a rootstock. It was named after German botanist and naturalist Georg Heinrich Wilhelm Schimper (1804-1878). The Malvaceae family includes Sida schimperiana. It's a perennial with a thick rhizome that pocked several procumbent or upright repeatedly forked branches 6-12 in long leaf stalk from the summit. Prenatal abortion, intestinal worms, amoebic dysentery, cough, influenza, and liver illness are treated with this plant species (Carmichale *et al*., 1987) [9]. In vitro, this plant has anticancer properties against gastric cancer (Lin *et al*., 2007 and Lin *et al*., 2005). The plant leaves were also thought to have anti-inflammatory and anticancer properties, according to Benjumea *et al*., 2016 [12]. The few phytochemical substances discovered in S. acuta were alkaloids (cryptolepine, ephedrine, and vasicine), saponosides, coumarins, steroids, phenolic compounds such as scopoletin, loliolid, 4-ketopinoresinol, evofolin-A and B, polyphenol, sesquiterpene, tannins, and flavonoids. Its efficacy against BT-549 breast cancer (Fadeyi *et al*., 2013) [14] and human hepatoma cells (HepG-2) (Pieme *et al*., 2010) [13] has previously been documented. Sida schimperiana has been examined for its anticancer and cytotoxic effects, but no previous evidence on its anticancer, cytotoxic, or apoptotic activity has been published. As a result, we investigated the potential anticancer activity of methanol infusion and decoction extracts from Sida schimperiana callus on MCF-7 breast cancer cell line in this study.

Materials and methods

Collection of Plant material

S.Schimperiana Hochst. ex A. rich were found in the village of Keeranur in the Pudukottai District of Tamil Nadu. Rapinat Herbarium Trichy (RTH) helped identify the plant specimen (Plant specimen No 29849) at St. Joseph's College (Autonomous). The callus culture sample was gently removed with the help of forcipes and we isolated the dried sample. The callus was cleaned, wiped with blotting paper, and stretched out in the shade at room temperature. The tissue blender was used to grind the shade dried samples

into a fine powder. The powdered samples were then kept in the refrigerator until they were needed again.

Cell culture and Media treatment

In this work, the MCF-7 breast cancer cell line was employed. Cells were cultivated and maintained at 37°C in a humidified incubator with a 5% CO2 environment. The culture medium for MCF-7 cell culture was DMEM supplemented with 10% FBS, 1 mM glutamine, 1% nonessential amino acids, 100 units ml–1 penicillin, and 100 mg ml–1 streptomycin. Concentrated stock solutions of extracts were produced in dimethyl sulfoxide (DMSO) before to the experiment, and stock solutions were diluted to the appropriate quantities using DMEM medium. The maximum concentration of DMSO in the wells was 0.1 percent (v/v), a value that had no effect on cell growth and was used as a control in all tests. In a sterile 24-well plate, cells were sown at a density of 5 104 cells/well and left to adhere overnight. The cells were treated with varied concentrations (25, 50, 75, 100, 150 g ml–1) of ethanol (infusion and decoction) extracts from S. Schimperiana callus and incubated for 24 and 72 hours under the same circumstances. Solvent controls were treated with equal amounts of DMSO as used for sample preparation to avoid the effect of DMSO on cell growth and death (in general 0.1 percent DMSO). For each period of the cell line, growth media control was also conducted in parallel.

Results and Discussion

The MTT experiment of *S. schimperiana* ethanol extract reveals that all doses have anticancer activity. We determined the IC50 values for the human breast cancer MCF-7 Cell line with sample concentrations of 25 g/ml, 50 g/ml, 75 g/ml, 100 g/ml, and 150 g/ml, respectively. MTT (3-(4,5-dimethyl thiazolyl)-2,5-diphenyl-tetrazolium bromide) is reduced to purple formazan product by mitochondrial dehydrogenase in the MTT experiment. These findings demonstrated that the leaf extracts caused morphological alterations and cell shrinkage in breast cancer cell lines, leading to cell death. (Plate – 1, Table –1-2, Fig.2) shows the IC50 values of ethanol extracts of S. Schimperiana leaf against (MDAMB231) breast cancer cell lines. The use of herbal medicines in cancer treatment has been attention, in recent years, because of their diverse Phyto-metabolic contents and numerous biological activities. In the selected extracts of the plant, the primary phytochemical investigation confirmed the existence of secondary metabolites. Many biological and therapeutic effects have been identified for these secondary metabolites. Phyto-constituents were measured using a variety of biochemical techniques. Ethanol extract contained phenols and flavonoids. We used ethanol extract to investigate the presence of glycosides (Prabhu and Ramar 2018). The research conducted on the phytochemical activity of *S. schimperiana* leaf extracts is noteworthy. Among the various phytochemicals, phenolic compounds have attracted the attention of diverse industries, including pharmaceutical, health, food, and cosmetics. These chemicals are abundant in the plant kingdom and exist as natural antioxidants in our regular diet. Many human diseases get influenced by reactive oxygen species (ROS). Due to the harmful significance of free radicals in biological systems, radical scavenging activities are critical. Numerous secondary metabolites, including phenols, polyphenols, and flavonoids, function as antioxidants and scavengers. ROS easily mix and oxidize biomolecules including carbohydrates, proteins, and lipids, rendering them inactive and causing damage to cells, tissues, and organs, ultimately contributing to cancer growth. The presence of antioxidants demonstrates to break the free radical cycle by donating a hydrogen molecule, which confers an antioxidant effect. The presence of antioxidants in the extract would cause ferri cyanide Fe3+ to get reduced to ferro cyanide Fe2+ by donating an electron, as detected by spectrophotometry at 570 nm.

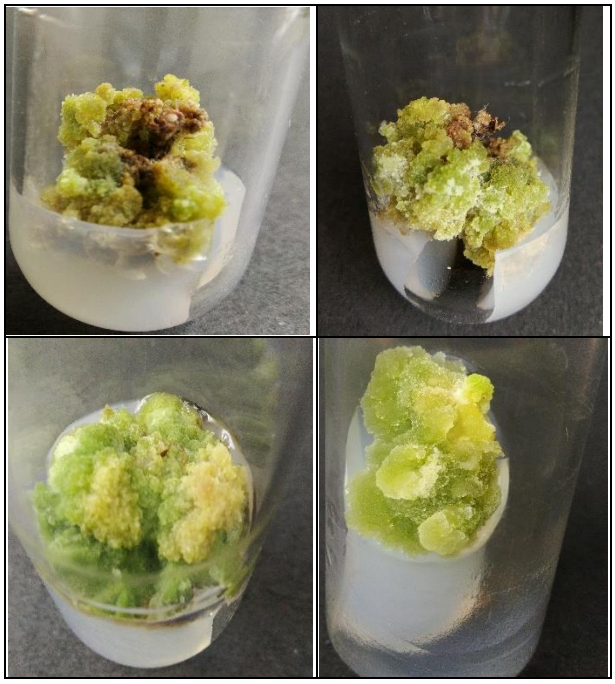


Plate 1: Different concretion and combination of Plant growth Regulators callus induction in *S. schimperiana* harvested Callus sample

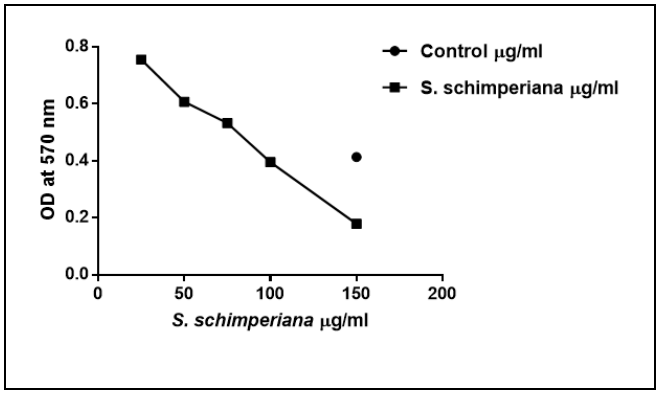
Anti-cancer cell line activity of *S. schimperiana* (Breast Cancer)

Table 1: Control Mean OD value: 0.414

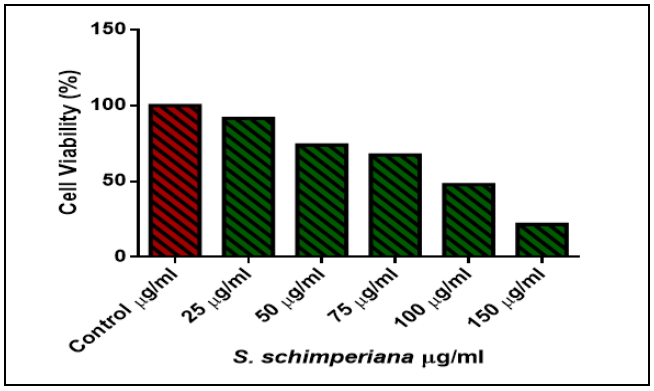
S. No	Tested sample concentration (µg/ml)	OD Value at 570 nm
1.	Control	100
2.	25 µg/ml	0.756
3.	50 µg/ml	0.608
4.	75 µg/ml	0.553
5.	100 µg/ml	0.396
6.	150 µg/ml	0.179

Table 2: OD Value at 570 nm

S. No	Tested sample concentration (µg/ml)	Cell viability %
1.	Control	100
2.	25 µg/ml	91.51
3.	50 µg/ml	73.93
4.	75 µg/ml	67.33
5.	100 µg/ml	47.81
6.	150 µg/ml	21.51



Graph 1: Cell viability for anticancer activity



Graph 2: Cell viability % in various concentration

Table 3: 3IC_{50} Value of tested sample: 82.5 µg/ml

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		1.916
HillSlope		-3.473
IC_{50}		82.50
Std. Error		
LogIC50		0.03392
HillSlope		0.9850
95% Confidence Intervals		
LogIC50		1.809 to 2.024
HillSlope		-6.607 to -0.3388
IC50		64.35 to 105.8
Goodness of Fit		
Degrees of Freedom		3
R square		0.9487
Absolute Sum of Squares		298.8
Sy.x		9.981
Number of points		
Analyzed	1	5

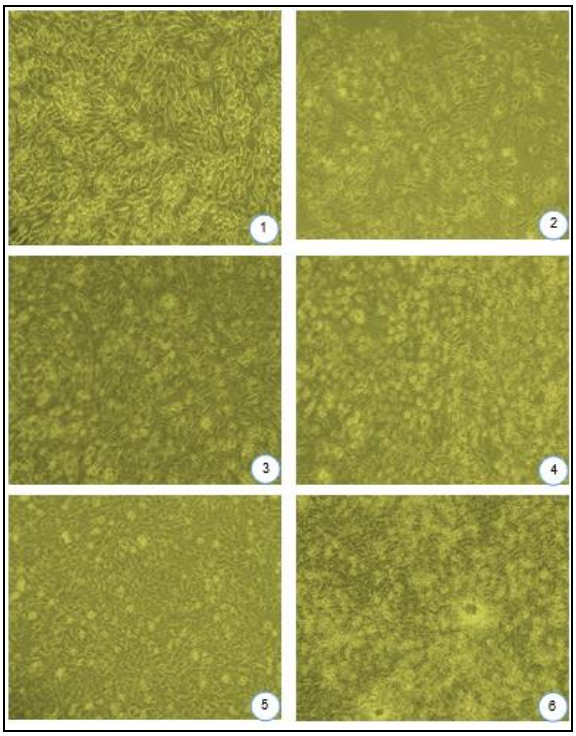


Fig 1: Control, Fig 2: 25 µg/ml, 91.51 cell viability %, Fig 3: 50µg/ml 73.93 cell death,
Fig 4: 75 µg/ml 67.33, Fig 5: 100 µg/ml 47.81, Fig 6: 150 µg/ml 21.51

Plate 1: Anticancer Cell line activity of *S. schimperiana* MDMBA 231 breast cancer

Conflict of Interest
The authors declare no conflict of interest.

Acknowledgements
The authors wish to express their gratitude to National College (Autonomous), Trichy.

References
1. Kultur S. Medicinal plants used in Kırklareli Province. Journal of Ethnopharmacol,2007:111(2):341-364.
2. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics. CA Cancer J Clin,2000:50(1):7-33.
3. Qi Y, Liao F, Zhao C, Lin Y, Zuo M. Cytotoxicity, apoptosis induction, and mitotic arrest by a novel podophyllotoxin glucoside, 4DPG, in tumor cells. Acta Pharmacol Sin,2005:26(8):1000-1008.
4. Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. J Nat Prod,1997:60(1):52-60.
5. Altobelli E, Lattanzi A. Breast cancer in European Union: An update of screening programmes as of Int J Oncol,2014:45:1785-1792.
6. Enguita M, Fortes P. Targeting the insulin- like growth factor pathway in hepatocellular carcinoma. World J Hepatol,2014:6:716-737.
7. Roberts H. Safety of Herbal Medicinal Products in Women with Breast Cancer," Maturitas,2010:66:4:363-369.
8. Wong LY, Leung PC, Tang JL, Mercer SW. Use of Dietary Supplements by Breast Cancer Patients Undergoing Conventional Cancer Treatment," Patient Prefer Adherence,2010:4:407-414.
9. Carmichale JWG, Degraff AF, Gazdar JDMinna and J. B. Mitchell, "Evaluation of a Tetrazolium Based Semi-automated Colorimetric Assay: Assessment of Chemo-Sensitivity Testing, Cancer Research,1987:47(4):936
10. Lin HH, Chen JH, Kuo WH, Wang CJ. Chemopreventive Properties of *Hibiscus Sabdariffa* L. on Human Gastric Carcinoma Cells through Apoptosis Induction and JNK/p38 MAPK Signaling Activation, Chemico Biological Interactions,2007:165(1):59-75.
11. Lin HH, Chen JH, Kuo WH, Wang CJ. *Hibiscus* Polyphenol-Rich Extract Induces Apoptosis in Human Gastric Carcinoma Cells via p53 Phosphorylation and p38 MAPK/FasL Cascade Pathway, Molecular Carcinogenesis,2005:43(2):86-99.
12. Benjumea DM, Gomez-Betancur IC, Vasquez J, Alzate F, Garcia-Silva A, Fontenla JA. Neuropharmacological effects of the ethanolic extract of *Sida acuta*. Revista Brasileira de Farmacognosia,2016:26(2):209-215.
13. Pieme CA, Penlap VN, Ngogang J, Costache M. *In-vitro* cytotoxicity and antioxidant activities of five medicinal plants of Malvaceae family from Cameroon. Environmental Toxicology and Pharmacology,2010:29:223-228.
14. Fadeyi SA, Fadeyi OO, Adejumo AA, Okoro C, Myles EL. *In vitro* anticancer screening of 24 locally used Nigerian medicinal plants. BMC Complementary and Alternative Medicine,2013:13(1):79.
15. Prabhu V. and Ramar K. Phytochemical screening by UV-Vis, FT-IR and GC-MS spectroscopic analysis of leaf and callus extracts of medicinal plants *Sida schimperiana* Hochst. ex A. rich, 2018, 7(11).