

How to Cite:

Kalyani, V. (2022). In vitro anticancer activity of *Semecarpus anacardium* on MCF-7 human breast cancer cells. *International Journal of Health Sciences*, 6(S4), 5882–5889. <https://doi.org/10.53730/ijhs.v6nS4.9323>

In vitro anticancer activity of *Semecarpus anacardium* on MCF-7 human breast cancer cells

Veeraveni Kalyani

Department of Zoology, Telangana Tribal Welfare Womens Degree & PG College
Thukkuguda, Mankhal, Hyderabad, Rangareddy, Telangana-501359, India

Abstract---*Semecarpus anacardium* (SA) is a plant native to India that is eaten as a fresh vegetable on a daily basis. The effects of SA therapy on human breast cancer MCF-7 cell lines were investigated in this study. Maceration with various solvents was used to prepare the crude extract of flavonoid fraction from SA bark, and fractionation was done using TLC and column chromatography techniques. MTT assay was used to assess the effects of the isolated flavonoid fraction of SA bark on cell viability. SA decreased MCF-7 cancer cell viability in a concentration-dependent manner, with IC₅₀ values of 24.18±1.20 µg/mL and 12.08±1.18 µg/mL after 24 and 48 hours of treatment, respectively. Finally, SA flavonoid fraction effectively reduced MCF-7 cells, implying that SA flavonoid fraction has anticancer potential against MCF-7 cells and may be useful in the prevention and treatment of breast cancer.

Keywords---MCF-7, *Semecarpus anacardium*, anticancer, TLC, column.

Introduction

Medicinal and culinary plants are increasingly being studied for their ability to boost health and prevent chronic diseases such as Alzheimer's disease (Bui and Nguyen 2017), diabetes (Liu et al. 2014), hyperlipidemia (Helmy et al. 2017), and cancer (Bui and Nguyen 2017). (Buranrat et al. 2017a,b). Many plants from various families have been used in India to treat or prevent cancers, such as liver cancer (Buranrat et al. 2017a), bile duct cancer (Senggunprai et al. 2016), colon cancer (Manosroi et al. 2014), cervix cancer (Kitdamrongtham et al. 2013), and breast cancer (Kitdamrongtham et al. 2013). (Buranrat et al. 2017b). *Semecarpus anacardium*, also known as the marking nut tree, phobi nut tree, and varnish tree, is a native of India that may be found from the outer Himalayas to the Coromandel Coast and belongs to the Anacardiaceae family. It's a close relative of the cashew.

Breast cancer is one of the most commonly fatal diseases in the world, and death is caused by metastasis to distant organs and the formation of secondary tumours, not by the main tumour. A fresh method for curing this cancer is urgently required. Because there is little information on the effects of CA on cancer cells, this study focused on the anti-cancer and antimigratory effects of CA extract on MCF-7 human breast cancer cells. This study looked at ROS production, caspase 3 activity, mitochondrial transmembrane potential, apoptotic and proliferative-related protein, and MMP protein expression to learn more about the underlying mechanism(s) that mediate CA leaf extract activity. The discoveries could help researchers locate new plants and phytochemicals that could be utilised to treat breast cancer.

Ayurvedic medicine uses several portions of *Semecarpus anacardium* for boosting sexual potency, raising sperm count, healing digestive system disorders, regulating phlegm, and causing abortion. The red-orange portion is gathered and sun-dried. It is eaten after it has been semi-dried. It is also dangerous if not purified, and the oil extracted from its seeds can cause blisters and painful lesions. Herbal plants have been shown to effectively lower protein-related cell proliferation while also increasing protein-related cell death, suggesting that they could be useful in cancer prevention and treatment. The effect of SA on other cancer types is less obvious, and its inhibitory activity, potency, and mechanism(s) of action should be investigated further.

There was no attempt to examine *Semecarpus anacardium* plant components as a source of cancer medicine, according to a review of the literature. However, some research into its additional therapeutic benefits has been conducted. As a result, people are becoming more interested in these herbs, especially since they are being used successfully by Indian tribes. As a result, it's worth looking into this plant further as a potential source of cancer treatment drugs. As a result, the current study was conducted to assess *Semecarpus anacardium*'s anticancer properties in MCF-7 human breast cancer cell lines.

Materials and Methods

Collection and Identification of Plant Materials

Semecarpus anacardium (SA) bark was taken fresh and healthy from the forest of Warangal district, Telangana state, India. The voucher specimens were all kept in the same place. Plant components were collected, cleaned thoroughly under running tap water, dried in the shade, and then triturated into fine powders using an electric grinder. This powder was kept at room temperature in airtight brown bottles.

Preparation of Plant Extracts

The powdered plant was extracted using various organic solvents in order of increasing polarity (hexane, chloroform, ethyl acetate, acetone and methanol). Separately, 500 grams of powder were macerated in 1500 mL hexane for three days with intermittent shaking. After that, it was filtered twice: once with muslin cloth and then again with Whatman no.1 filter paper. The residue was extracted

twice with the same fresh solvent, and all of the filtrates were combined. The resultant residue was air dried before being extracted using chloroform, ethyl acetate, and acetone in the same way as the hexane extraction was done. Finally, the solvent was evaporated from each filtrate using a rotary evaporator at low pressure and temperature. Each extract's yield was weighed and stored at 4 °C until it was utilised.

Isolation and purification of active phytochemicals

The antitumor efficacy of the crude extracts produced was investigated. After assessing the anticancer activity of crude extracts *in vitro*, it was decided to pursue additional research and look for the active phytochemical responsible for the anticancer action. The fractionation of active phytochemicals was done using TLC and column chromatography.

Thin Layer Chromatography

TLC (Thin Layer Chromatography) is a type of chromatography in which the layers are separated by a thin layer. TLC was carried out on silica gel, F254 plates with an aluminium backing. The active crude extract of SA was dissolved in a suitable solvent and spotted onto the TLC plate at a distance of 1 cm from the bottom of the plate in a volume of 2 μ l. After drying the spots with a warm current of air, the plate was developed in a saturated glass TLC tank with the appropriate solvent system. TLC plates were exposed to iodine vapours in order to visualise the spots in the TLC. For each chromatogram, R_f values were computed. On the basis of clear separation on a TLC plate, the solvent system for column chromatography was chosen.

Column Chromatography

A column chromatography flask was used to make the column. To keep the silica from escaping the column, glass wool was added at the bottom of the flask. The concentrated active extract was chromatographed on a silica gel column using the appropriate solvent solution, and fractions were collected and monitored using TLC. The collected fractions with similar R_f values were pooled and evaporated to produce a residue, which was then examined for anti-cancer efficacy *in vitro*. The rotary evaporator was used to concentrate the most active pooled fractions, which were then assessed for phytochemical research.

Anticancer Activity Research *in Vitro*

Culture of cells

The MCF-7 cancer cell line was bought from NCCS, Pune, and the cells were kept in DMEM supplemented with 10% FBS and antibiotics penicillin/streptomycin (0.5 mL⁻¹), at 37°C, in a 5 percent CO₂/95 percent air environment. DMEM (Dulbecco's modified Eagles media), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS), and Fetal Bovine Serum (FBS) were all acquired from Sigma Chemicals Co. (St.

Louis, MO). Eppendorf India provided 25 cm² and 75 cm² flasks, as well as 96 well plated flasks.

MTT assay (Methyl Tetrazolium Assay)

MTT Assay is a colorimetric assay that assesses mitochondrial succinate dehydrogenase's reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). The assay is based on the quantity of cells present as well as the premise that tetrazolium is not reduced by dead cells or their products. MTT enters the cells and travels to the mitochondria, where it is transformed into insoluble dark purple formazan crystals. The cells are subsequently dissolved in DMSO, and the solubilized formazan reagent is spectrophotometrically quantified at 570 nm.

The MTT Assay was used to assess cell viability in three independent trials with six different chemical doses in triplicates. To determine the viability of cells in suspension, cells were trypsinized and the tryphan blue assay was performed. Cells were counted using a haemocytometer and seeded at a density of 5.0×10^3 cells per well in 100 l media in 96 well plate culture medium. Remove the old media after incubation and replace it with 100 ml of fresh media containing varying concentrations of the test substance in the represented wells of 96 plates. After 48 hours, discard the medication solution and replace it with fresh media containing MTT solution (0.5 mg/mL-1) in each well, which was incubated at 37°C for 3 hours. Precipitates occur at the conclusion of the incubation period as the MTT salt is reduced to chromophore formazan crystals by cells with metabolically active mitochondria. On a microplate reader, the optical density of solubilized crystals in DMSO was determined at 570 nm. The following formula was used to compute the percentage of growth inhibition.

$$\% \text{ Inhibition} = \frac{100 (\text{Control} - \text{Treatment})}{\text{Control}}$$

The IC₅₀ value was determined by using linear regression equation i.e. $y = mx + c$. Here, $y = 50$, m and c values were derived from the viability graph (Figure-1 and 2).

Results and Discussions

Anticancer of activity of SA flavonoid fraction

The alkylating agent, cisplatin, was measured in the crude extract. MCF-7 human breast cancer cells were used to test the impact of SA on cell viability. After treating MCF-7 cells for 24 and 48 hours, cytotoxic effects revealed that cell proliferation was significantly reduced, with IC₅₀ values of 24.18 ± 1.20 and 12.08 ± 1.18 µg/mL for MCF-7 cells, respectively (Table-1). Surprisingly, the amount of SA needed to stop MCF-7 cells from growing was lower than the amount needed to stop them from multiplying. Table-1 shows the percentage of MCF-7 human breast cancer cells viability after incubation with SA fraction at various concentrations for 24 and 48 hours, along with IC₅₀ values.

Table-1. Percentage of MCF-7 human breast cancer cells viability due to incubation of SA fraction in different concentration at 24 hours and 48 hours with IC₅₀ values

Concentration (μg)	% Viability		IC ₅₀ (μg)	
	24 h	48 h	24 h	48 h
5	80.34	71.80		
10	68.43	58.42		
25	51.6	40.09	24.18 ± 1.20	12.08 ± 1.18
50	38.50	23.02		
100	25.90	21.30		
Untreated (0)	100	100		

Figures 1 and 2 show the determination of IC₅₀ values using a linear regression equation in 24 and 48 hour incubations of MCF-7 cancer cell lines with *Semecarpus anacardium* fraction. When employed at a high concentration (100 $\mu\text{g}/\text{mL}$), the SA fraction, which is recognized as flavonoid, generates significantly higher MCF-7 viability percentage in 48 hours (21.30 $\mu\text{g}/\text{mL}$) than in 24 hours (25.90 $\mu\text{g}/\text{mL}$) incubation. When compared to the untreated control group, modest concentrations of the SA fraction (25 and 50 $\mu\text{g}/\text{mL}$) did not boost MCF-7 cell viability. Figures 3 and 4 show the effects of SA flavonoid fraction on MCF-7 cancer cell proliferation after 24 and 48 hours of incubation.

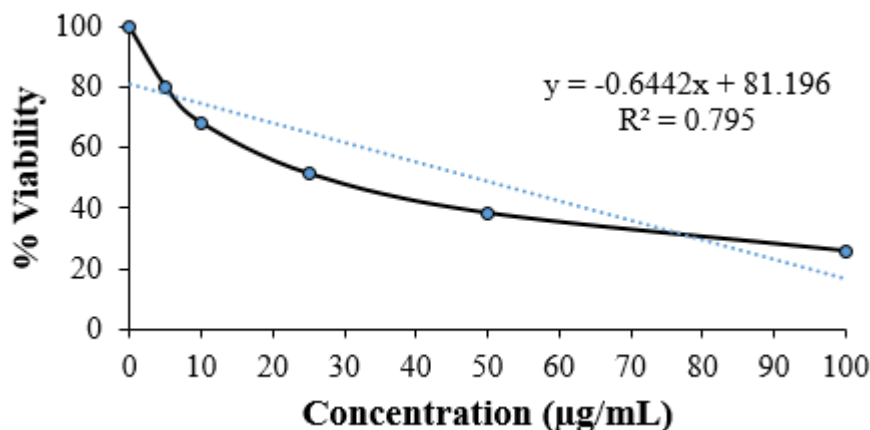


Figure-1. The determination of IC₅₀ value by using linear regression equation in 24 hours incubation of MCF-7 cancer cell lines with *Semecarpus anacardium* fraction

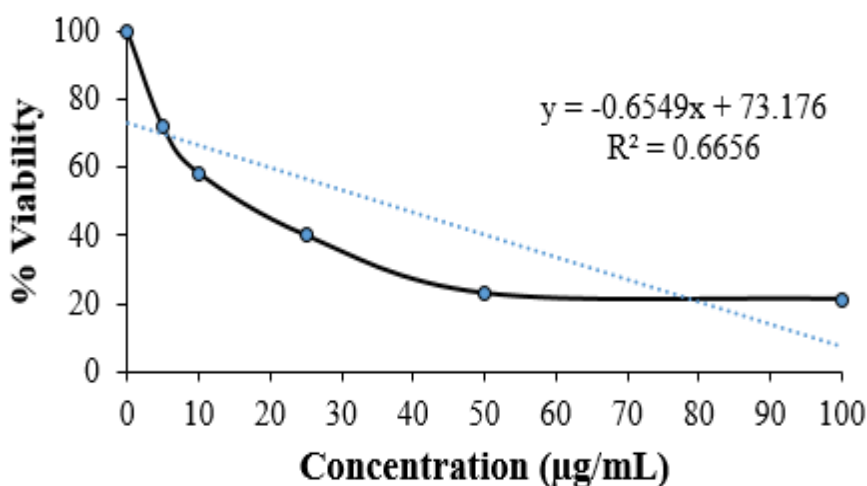


Figure-2. The determination of IC₅₀ value by using linear regression equation in 48 hours incubation of MCF-7 cancer cell lines with *Semecarpus anacardium* fraction

Phytochemicals such as careyagenolide, careaborin-I, -amyrin, triterpanoid sapogenols, taraxerol, -sitosterol, flavonoids, sterols, and coumarin have been found in the leaves, bark, and seeds of SA (Basak et al. 1976). Many chronic disorders, including cancer, have been linked to the active chemicals in SA. According to our findings, the SA bark extract includes a high concentration of flavonoids components, and numerous research have shown that plants high in flavonoid and phenolic compounds have anticancer properties in vitro. Following this pattern, the SA extract was discovered to significantly decrease breast cancer cell growth in a concentration- and time-dependent manner, as well as inhibit MCF-7 cell growth with a low IC₅₀.

A technique for limiting cancer metastasis to distant organs is to produce anticancer medications and medical items that impede cancer cell migration (Steeg and Theodorescu 2008). MMPs are protease enzymes that break down the extracellular matrix integrity in the microenvironment, which is an important stage in cancer metastasis (Folgueras et al. 2004). SA significantly reduced MCF-7 cell viability in the MTT experiment at doses of 50 and 100 µg/mL, according to our findings.

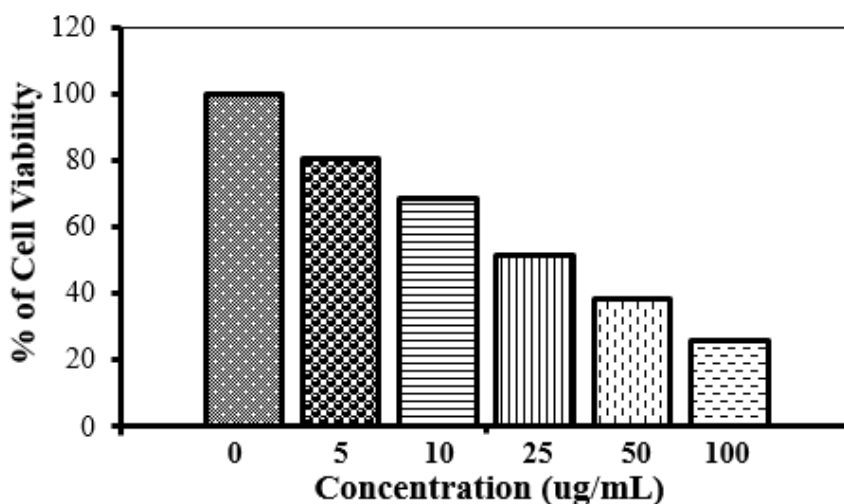


Figure-3. Effects of SA flavonoid fraction on proliferation of MCF-7 cancer cells during 24 hours incubation. Data represent mean $\pm$ SEM averaged from three independent experiments. * $p < 0.05$ different from control. SA, *Semecarpus anacardium*

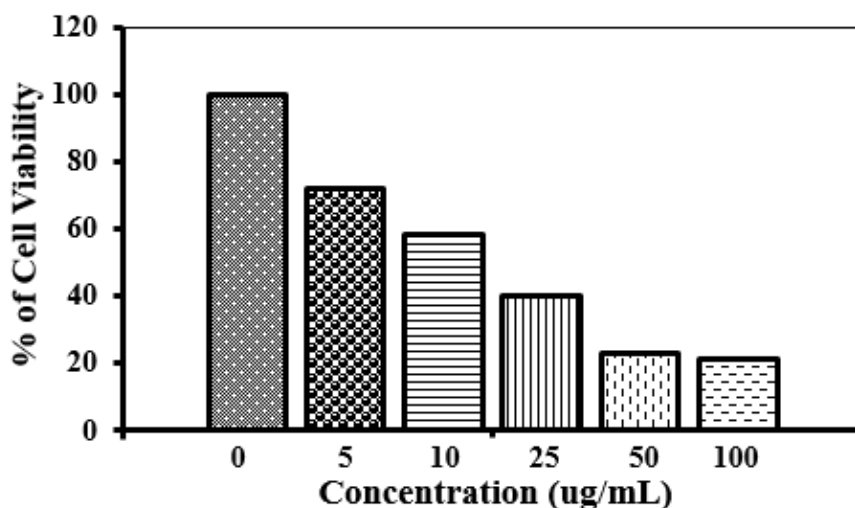


Figure-4. Effects of SA flavonoid fraction on proliferation of MCF-7 cancer cells during 48 hours incubation. Data represent mean $\pm$ SEM averaged from three independent experiments. * $p < 0.05$ different from control. SA, *Semecarpus anacardium*

Conclusion

This study has demonstrated that SA bark extract which contains flavonoid fraction inhibited the proliferation of MCF-7 cells. These findings provide a theoretical basis for SA and other Indian medicinal plants to be further investigated as sources of drugs to treat cancer and other diseases; however, the precise mechanisms of action need to be explored.

Acknowledgments

The authors are grateful to Department of Zoology, Tribal Welfare College and Synteny Life Sciences Pvt Ltd for providing all of the research resources.

References

- Basak A, Banerjee S, Bose L, Basu K (1976) Chemical examination of the leaves of *Careya arborea*. *J. Indian Chem* 53:639–640.
- Bui TT, Nguyen TH (2017) Natural product for the treatment of Alzheimer's disease. *J Basic Clin Physiol Pharmacol* 28:413–423. <https://doi.org/10.1515/jbcpp-2016-0147>.
- Buranrat B, Mairuae N, Konsue A (2017b) *Cratoxy formosum* leaf extract inhibits proliferation and migration of human breast cancer MCF-7 cells. *Biomed Pharmacother* 90:77–84. <https://doi.org/10.1016/j.biopha.2017.03.032>.
- Folgueras AR, Pendas AM, Sanchez LM, Lopez-Otin C (2004) Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *Int J Dev Biol* 48:411–424. <https://doi.org/10.1387/ijdb.041811af>.
- Helmy SA, Morsy NFS, Elaby SM, Ghaly MAA (2017) Hypolipidemic effect of *Moringa oleifera* lam leaf powder and its extract in dietinduced Hypercholesterolemic rats. *J Med Food* 20:755–726. <https://doi.org/10.1089/jmf.2016.0155>.
- Kitdamrongtham W, Manosroi A, Akazawa H, Gidado A, Stienrut P, Manosroi W, Lohcharoenkal W, Akihisa T, Manosroi J (2013) Potent anti-cervical cancer activity: synergistic effects of Thai medicinal plants in recipe N040 selected from the MANOSROI III database. *J Ethnopharmacol* 149:288–296. <https://doi.org/10.1016/j.jep.2013.06.037>.
- Liu YN, Jung JH, Park H, Kim H (2014) Olive leaf extract suppresses messenger RNA expression of proinflammatory cytokines and enhances insulin receptor substrate 1 expression in the rats with streptozotocin and high-fat diet-induced diabetes. *Nutr Res* 34: 450–457. <https://doi.org/10.1016/j.nutres.2014.04.007>.
- Manosroi A, Akazawa H, Akihisa T, Jantrawut P, Kitdamrongtham W, Manosroi W, Manosroi J (2014) In vitro anti-proliferative activity on colon cancer cell line (HT-29) of Thai medicinal plants selected from Thai/Lanna medicinal plant recipe database "MANOSROI III". *J Ethnopharmacol* 161:11–17. <https://doi.org/10.1016/j.jep.2014.11.038>.
- Senggunprai L, Thammaniwit W, Kukongviriyapan V, Prawan A, Kaewseejan N, Siriamornpun S (2016) *Cratoxylum formosum* extracts inhibit growth and metastasis of Cholangiocarcinoma cells by modulating the NF-kappaB and STAT3 pathways. *Nutr Cancer* 68: 328–341. <https://doi.org/10.1080/01635581.2016.1142580>.
- Steeg PS, Theodorescu D (2008) Metastasis: a therapeutic target for cancer. *Nat Clin Pract Oncol* 5:206–219. <https://doi.org/10.1038/ncponc1066>.
- Suryasa, I. W., Rodriguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, 5(1), i-v. <https://doi.org/10.53730/ijhs.v5n1.2864>
- Thaib, P. K. P., & Rahaju, A. S. (2022). Clinicopathological profile of clear cell renal cell carcinoma. *International Journal of Health & Medical Sciences*, 5(1), 91-100. <https://doi.org/10.21744/ijhms.v5n1.1846>