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SYNERGISTIC ANTICANCER ACTIVITY OF CATHARANTHUS *ROSEUS* LINN. AND SOLANUM *LYCOPERSICUM* LINN.

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ABSTRACT

Breast cancer is a condition where malformed breast cells proliferate and develop into tumours. Tumours can become deadly if they proliferate throughout the body and are not treated. So, this study explores the synergistic anti-cancer activity derived from the combination of *Catharanthus roseus* (commonly known as Madagascar periwinkle), a plant source of vinblastine and vincristine, potent anti-cancer alkaloids, with *Solanum lycopersicum* (tomato), a rich reservoir of lycopene, a recognized anti-cancer pigment. The rationale behind this investigation lies in the potential enhancement of anti-cancer effects through the complementary action of these bioactive compounds.

The research involves extraction of *Catharanthus roseus* and *Solanum lycopersicumin* in respective solvents. Subsequently, combined ratio of both plant extract are subjected to evaluate for synergistic activity on breast cancer cell lines. Preliminary findings indicate a notable increase in anti-cancer activity when combined chemical constituent from both extract which is more prone for anticancer activity, suggesting a synergistic interaction. This synergy may be attributed to complementary mechanisms of action to achieve hypothesis.

Combination therapy, a treatment modality that combines two or more therapeutic agents, is a cornerstone for cancer therapy. The amalgamation of anti-cancer drugs enhances efficacy compared to the mono-therapy approach because it targets key pathways in a characteristically

synergistic or an additive manner. This approach potentially reduces drug resistance, while simultaneously providing therapeutic anti-cancer benefits, such as reducing tumour growth and metastatic potential, arresting mitotically active cells, reducing cancer stem cell populations, and inducing apoptosis.

Keywords: *Catharanthus Roseus Linn.*, *Solanum Lycopersicum Linn.*, lycopene, vincristine, vinblastine, synergistic anticancer activity

INTRODUCTION:

Cancer remains a formidable health challenge worldwide, with a growing number of patients seeking effective therapeutic interventions. Increased incidence of cancer in recent years and its impact on different physical, mental, and social dimensions of human life have turned it to a major problem of the century. The incidence of this disease in developed countries varies from 1 to 2 percent, with almost 5% yearly increase in less developed countries. Meanwhile, breast cancer is the most prevalent type of malignant neoplasm among women with more than one million new cases per year. In India, breast cancer accounts for the major type of cancer among women with the incidence of 21.4 or 32%. Breast cancer is the most common type of cancer among women in the US with the incidence rate of 12.5%. The risk of an individual dying from breast cancer is 1-in-

35. Regarding the importance of this issue, this study sought to investigate breast cancer and its associated factors **[1]**.

Breast cancer is the most common type of cancer and the second leading cause of death. This disease is the primary cause of mortality among women aged 45–55 years, and is the second leading cause of cancer-induced death. The incidence of breast cancer is almost 1-in-8 women, requiring complete tissue removal,

chemotherapy, radiotherapy, and hormone therapy most of the time. Breast cancer is a type of tissue cancer that mainly involves inner layer of milk glands or lobules, and ducts (tiny tubes that carry the milk) **[2]**. The primary risk factors of cancer include age, high hormone level, race, economic status, and iodine deficiency in diet. Breast cancer is a multi- stage disease, in which viruses play a role in one stage of this pathogenic process. In general, viruses are involved with different cancer types **[3]**.

Medicinal plants have been traditionally used in folk medicine for centuries as natural healing remedies with significant proven therapeutic effects in many areas including prevention of anticancer activity, cardiovascular diseases, anti-inflammatory, antimicrobial activity. In addition, the emergence of resistance to cancer chemotherapy has forced researchers to turn
to natural products of plant and marine origin
[4]. Although many compounds isolated from plants are being strictly tested for their anticancer properties, it is recognized that the beneficial effects of combined plant extract due to a complex interplay of the composite mixture of chemical constituent present in the more than one plant (additive/synergistic and/or antagonistic) rather than single plant extract **[5]**.

In this context, the present research investigates the synergistic anticancer potential of two potent botanical agents, *Catharanthus roseus* Linn. and *Solanum lycopersicum* Linn., on breast cancer cells **[6]**. The primary objective of this study is to explore the synergistic effects resulting from the combination of these two plant-derived compounds, shedding light on their collective efficacy against breast cancer **[7]**. Despite the escalating prevalence of cancer, there exists a research gap concerning the simultaneous application of *Catharanthus roseus* and *Solanum lycopersicum* as anticancer agents, making this investigation particularly significant **[8]**.

The study focuses on breast cancer cells, emphasizing the need for novel therapeutic approaches to address this prevalent form of cancer **[9]**. Through rigorous experimentation and analysis, we aim to contribute valuable insights into the synergistic mechanisms underlying the combined anticancer activity of these botanical agents (*Catharanthus roseus* Linn. and *Solanum lycopersicum* Linn.) **[10]**.

Plant Profile:

a) Solanum *lycopersicum* Linn.: Taxonomic Classification: [11, 12]

- Kingdom: Plantae
- Subkingdom: Tracheobionta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Asteridae
- Order: Solanales
- Family: Solanaceae
- Genus: Solanum
- Species: *Solanum lycopersicum* Linn.

Figure1:Solanum*lycopersicum*Linn.

Chemical Constituents: [13, 14] Carotenoids such as Lycopene, Phytoene and Beta-carotene, Rich source of Vitamin C, Vitamin E, Luteine.

Solanum *lycopersicum* Linn. as an anticancer: [15]

Solanum lycopersicum Linn. contains lycopene pigment. Lycopene caused tumor cells to accumulate in the G0/G1 phase and undergo apoptosis. However, most cancer cells presented an increase in G2/M phase as the incubation time was prolonged. Several studies suggested lycopene decreased cell cycle related proteins, such

as cyclin D1, D3 and E, the cyclin dependent kinases 2 and 4, bcl-2, while decreased phospho-Akt levels

b) *Catharanthus roseus* Linn.: Taxonomic Classification: [16, 17]

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Gentianales
- Family: Apocynaceae
- Genus: Catharanthus
- Species: *Catharanthus. roseus* Linn.

Chemical Constituents: **[18, 19]**

Figure 2: Catharanthus *roseus* Linn

because of none separation of chromosomesVincristine, Vinblastine, Vindoline, Ajmalicine, Vinceine, Vincamine, Raubasin, Reserpine, Catharanthine, Vindesine, Vindeline, Tabersonine

Catharanthus roseus Linn. as an Anticancer: [20, 21]

Vinblastine inhibits the cell cycle of cancer cells. It binds with tubulin, and inhibits the formation of microtubules. Due to the inhibition, formation of microtubules takes place and cell cycle arrest in M phase during anaphase of mitosis. The chemical structure of vinblastine and vincristine are very similar, but their effects are not the same. Vinblastine is used to treat specific types of cancer, such as Hodgkin's disease, and Vincristine is used in the treatment of acute lymphoblastic leukemia.

MATERIALS AND METHOD:

- **Solanum *lycopersicum* Linn. (Sample Code - TL):**Collection: We have collected fruit of Solanum lycopersicum Linn. from Deur,Taluka-Koregoan, District- Satara.
- Pulp formation: The pulp is made by crushing fruits into crude tomato juice by using mixure **[22]**.
- Drying: The above pulp was taken in petridish and dried in hot air oven at 50-60°C for two days.
- Powdering: The pulp was reduced into fine size.
- Soxhlation: The fruit powder was subjected for extraction by using methanol at 55°C. (For 8 Hours daily for two days) **[23- 26]**
- Drying: We took 100 gm activated Magnesium sulphate as adsorbent, and placed into dessicator to dry the extract **[27]**.

B) Catharanthus *roseus* Linn. (Sample Code - VV):

- Collection: Leaves of Catharanthus roseus Linn. was collected.
- Drying: Leaves were shed dried for one month.
- Powdering: Dried leaves were finely grinded into powder.
- Soxhlation: The leaves powder was subjected for extraction by using methanol at 55°C. (For 8 Hours daily for five days) **[28, 29]**
- Drying: We took 100 gm activated Magnesium sulphate as adsorbent, and placed into dessicator to dry the extract.

C) Mixing of both extract (Sample code - VT):

Above Both extract mixed in 1:1 proportion and send for testing.

Table 1

Sample Code	Name of Extract
VV	*Catharanthus roseus* Linn.
TL	*Solanum lycopersicum* Linn.
VT	Mixture of both (1:1)

Figure 3: Collection of Plant

Figure 4: Soxhlation (VV)

Figure 5: Soxhlation (TL)

Figure 6: Drying of Sample VV

Figure 7: Drying of Sample TL

Phytochemical Analysis: [30-35]

A) Test for Glycosides:

1. **Keller killani test**:(Cardiac glycosides) the test solution with few drops of glacial acetic acid in 2 ml of ferric chloride solution and concentrated sulphuric acid is added from the sides of the test tube which shows the separation between two layers, lower layer shows reddish brown and upper layer turns bluish green in colour.
2. **Raymond's test**: Test solution treated with dinitrobenzene in hot methanolic alkali gives violet color.
3. **Legal's test**: The test solution treated with 1ml pyridine and 1ml sodium nitroprusside gives pink to red color appears.

B) Test for Alkaloids:

1. **Mayer's test**: Test solution treated with Mayer's reagent (Potassium mercuric iodide) gives cream colored
precipitate.

2.**Wagner's test**: The acidic test solution treated with Wagner's reagent (Iodine in potassium iodide) gives brown precipitate.

3. **Hager's reagent**: The acetic test solution treated with Hager's reagent (Saturated picric acid solution) gives yellow precipitate.

C) Test for Flavanoids:

1. **Ferric chloride test**: The test solution with few drops of ferric chloride solution shows intense green color.

2. **Shinoda test**: Test solution with few fragments of magnesium ribbon and concentrated hydrochloric acid shows pink to magenta red color.
3. **Zinc - Hydrochloric acid- reduction test**: Test solution with zinc dust and few drops of hydrochloric acid shows magenta red color.
4. **Alkaline reagent test**: Test solution

when treated with sodium hydroxidesolution shows increase in the intensity of yellow color which becomes colorless on addition of few drops of dilute acid.

5. **Lead acetate solution test**: Test solution with few drops of lead acetate solution (10% w/v) gives yellow precipitate.

B) Test for Steroids:

1. **Liebermann burchard test**: The test solution treated with few drop of acetic anhydride and mixed well. When concentrated sulphuric acid is added from the side of the test tube, it shows a brown ring at the junction of the two layer and the upper layer turns green.
2. **Salkowski's Test**: The second portion of solution above was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown color indicative of steroidring.

C) Test for Phenols:

1. **Ferric Chloride Test**: A small amount of the ethanolic extract was taken with 1 ml of water in a test tube and 1 to 2 drops of Iron III chloride (FeCl3) was added. A blue, green, red or purple color is a positive test.
2. **Lead acetate Test**: Plant extract is dissolved in 5mL distilled water +

3mL of 10% lead acetate sol. A white precipitate.

3. **Ellagic Acid Test**: Plant extract aqueous solution + 5% glacial acetic acid + 5% sodium nitrite solution A Solution turns muddy / Niger brown precipitate.
4. **Gelatin Test**: Plant extract is dissolved in 5 mL distilled water
+1% gelatine solution + 10% NaCl A white precipitate.

D) Test for Caretenoids:

1. **Caretenoid Test**: 1 g of each specimen sample was extracted with 10ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

Pharmacological Activity: [36]

The three different extract was send to Infinite Biotech Laboratory, Sangli for invitro anticancer activity.

a) Cell line:

MCF7 (Breast Cancer cell line)

b) Media:

DMEM with high glucose (Cat No- 11965-092), FBS (Gibco, Invitrogen)

Cat No-10270106 Antibiotic- Antimycotic 100X solution (Thermo fisher Scientific) Cat No-15240062Experimental Procedure (MTT Assay): [37-40]

1. Cells were incubated at a concentration of 1 × 104 cells/ml in culture medium for 24 h at 37°C and 5% CO2.
2. Cells were seeded at a concentration (100µl) 104 cells/well) in 100µl culture medium and 20, 40, 60, 80,
100 µg/ml of Samples into micro plates respectively (tissue culture grade, and 96 wells).
3. Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture.
4. Cell cultures were incubated for 24 h at 37°C and 5% CO_2 in CO_2 incubator.
5. After incubation, the medium was completely removed and Added 20µl of MTT reagent. (5mg/min PBS).
6. After addition of MTT, cells incubated for 4 hours at 37oC in CO_2 incubator.
7. Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark colored formazan by viable cells only.
8. After removing the medium completely. Added 200 µl of DMSO (kept for 10 min) and incubate at 37ºC (wrapped with aluminum foil).

9.

RESULTS:

i) Results of Phytochemical Test for Sample VV & TL: [41-44]

Table: 2 Results of Phytochemical Test

Sr. No.	Chemical Constituents	Tests	TL	VV	Observation
1.	Steroids	Salkowaski test	++	--	Present in TL,Partially in VV
		Liebermann Burchard test	++	++	
		Sulphur test			
2.	Glycosides	Balget's test			Present in TL Partially in VV
		Keller killiani test	++	--	
		Legal's test	++	++	
3.	Alkaloids	Wagner's test			Present in TL,Partially in VV
		Hager's test			
		Mayer's test	++	--	
4.	Flavonoids	Lead acetate	--	++	Partially present inTL and Moderate in VV
		Zinc hydrochloride	--	--	
		5% Ferric chloride test	++	--	
		Alkaline reagent	--	++	
		Shinoda Test	--	++	
5.	Phenol	Ferric chloride test	++	++	Present in Both
		Lead Acetate test	++	--	
		Ellagic Test	--	--	
		Gelatin test	++	++	
6.	Caretenoids	Caretenoid test	--	++	Present in VV

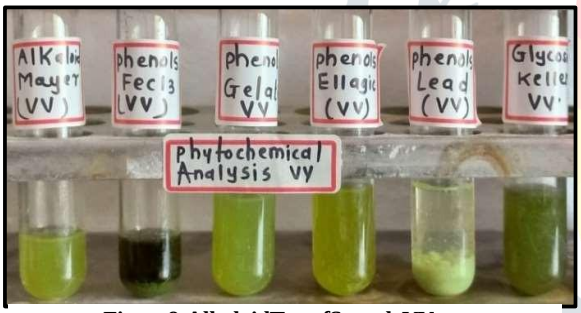

Figure8:AlkaloidTestofSampleVV

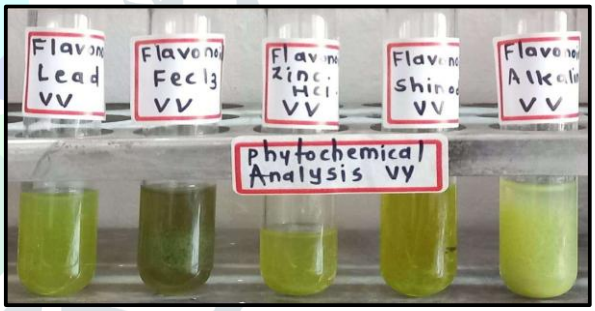

Figure 9: Flavanoid Test of Sample VV

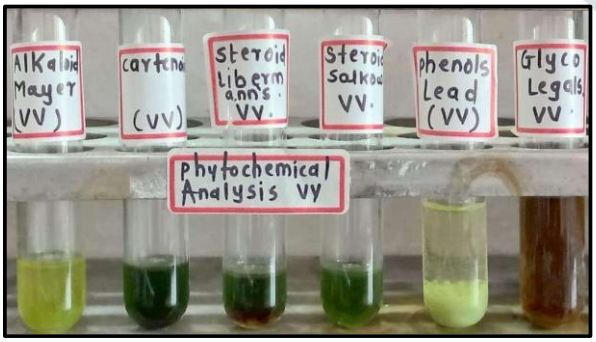

Figure 10: Steroidal Test of Sample VV

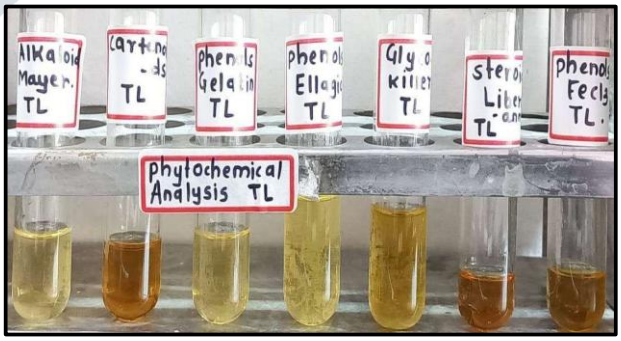

Figure 11: Alkaloid Test of Sample VV

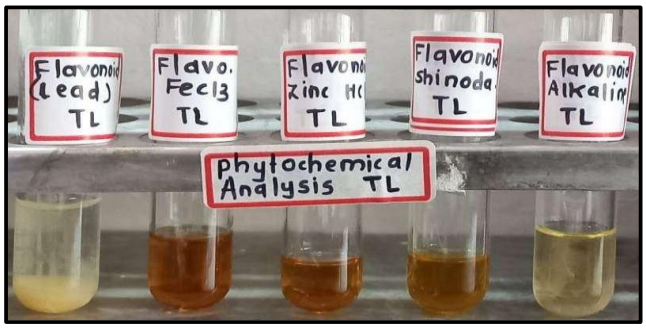

Figure 12: Alkaloid Test of Sample VV

ii) Anticancer Activity:

Table: 3 Anticancer activity of samples on Breast Cancer cell line [45-49]

S. No.	Sample Code	Conc. (ug/ml)	OD			Mean	% of Inhibition	% of Viability	IC50 (uL/ml)
1	Control		1.307			-	-	-	-
2	Standard	20	1.007	1.008	1.007	1.007	22.95%	77.05%	
	(%,Flurouracil)	40	0.891	0.890	0.891	0.891	31.82%	68.18%	49.23
		60	0.551	0.550	0.551	0.550	57.91%	42.08%	
		80	0.436	0.436	0.437	0.436	66.64%	33.35%	
		100	0.279	0.278	0.279	0.278	78.72%	21.27%	
3	VV	20	1.168	1.168	1.168	1.168	10.63%	89.36%	NE
		40	1.054	1.054	1.055	1.054	19.35%	80.64%	
		60	0.920	0.920	0.920	0.920	29.60%	70.39%	
		80	0.840	0.842	0.840	0.840	35.73%	64.26%	
		100	0.796	0.796	0.796	0.796	39.09%	60.90%	
4	TL	20	1.206	1.208	1.206	1.206	7.72%	92.27%	NE
		40	1.008	1.007	1.008	1.007	22.95%	77.04%	
		60	0.956	0.954	0.954	0.954	27.00%	72.99%	
		80	0.812	0.810	0.812	0.811	37.94%	62.05%	
		100	0.754	0.754	0.756	0.754	42.31%	57.68%	
5	VT	20	1.005	1.005	1.005	1.005	23.10%	76.89%	54.25
		40	0.920	0.922	0.920	0.920	29.60%	70.39%	
		60	0.825	0.825	0.825	0.825	36.87%	63.12%	
		80	0.710	0.712	0.712	0.711	45.60%	54.39%	
		100	0.626	0.626	0.627	0.626	52.10%	47.89%	

*NE- Not Evaluable

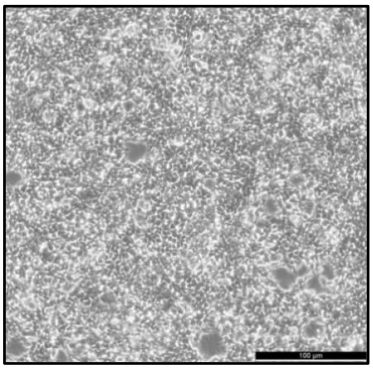

Figure 13: Control

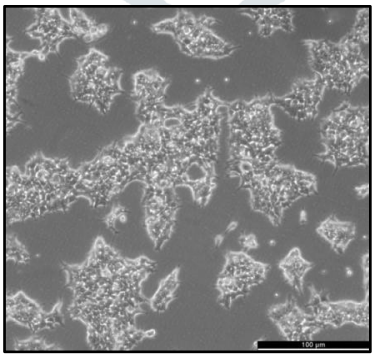

Figure 14: Standard

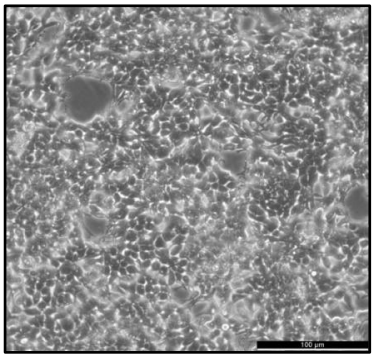

Figure 15: Effect of Sample VV

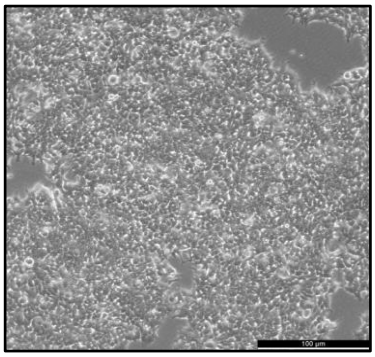

Figure 16: Effect of Sample TL

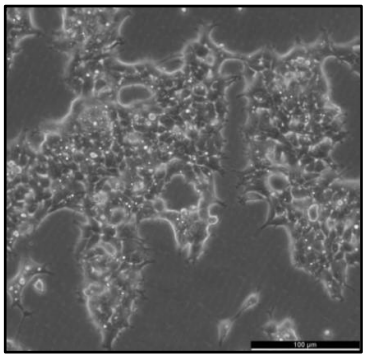

Figure 17: Effect of Sample VT

DISCUSSION:

This research holds the potential to pave the way for innovative and effective strategies in cancer treatment, benefiting the ever- increasing population affected by this devastating disease. At the differentConcentrations Sample Code VT showed High percent of inhibition and having good anticancer activity against MCF7 Breast Cancer cell line as compared to standard drug 5FU and sample code VV, TL shows the low percent of inhibition and shows the moderate anticancer activity. In this research work, we understood the synergistic potential of these plant-derived compounds opens avenues for developing novel and stable anti-cancer formulations that capitalize on their combined benefits. Further exploration of the underlying molecular mechanisms and in vivo studies is warranted to validate and optimize this synergistic approach for potential therapeutic applications in cancer treatment. As well as clinical trials to assess the safety and efficacy of the combined plant extracts in human subjects, moving towards potential therapeutic applications.

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DECLARATION

Conflict of interest

The authors state that there is no conflict of interest with this study.

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