

**IMMUNO ACTIVATING PHYTONCIDES SOAP: A TOPICAL APPROACH TO
STIMULATE NK CELL FOR CANCER PREVENTION*****Dr. L. V. Vigneshwaran, Mukesh Kumar C., Mithra K. C., Sandhiya V., Thirumal S., Siva Shankar B.**

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ABSTRACT

Cancer prevention through immune modulation has gained increasing attention in recent years. Natural Killer Cells play a crucial role in the early detection and destruction of tumor cells as part of the innate immune system. Phytoncides, volatile bioactive compounds released from plants, have been reported to possess antioxidant, anti-inflammatory, and immunomodulatory properties. This study focuses on the formulation of an immuno-activating phytoncides soap containing compounds such as Alpha-Pinene to explore its potential role in cancer prevention. The formulated soap was evaluated for physicochemical properties, antioxidant activity, anti-inflammatory activity, and in vitro anticancer activity using cancer cell lines. The findings suggest that phytoncide-based topical formulations may contribute to immune stimulation and provide supportive benefits in cancer prevention strategies.

KEYWORDS: Alpha -pinene, anti- cancer, anti- oxidant, anti- inflammatory.**1. INTRODUCTION**

One of the main causes of sickness and death in the world, cancer is typified by unchecked cell growth and the capacity of aberrant cells to infiltrate neighboring tissues and spread to other organs. The global cancer burden continues to rise due to a number of factors, including aging populations, environmental exposure, and lifestyle changes. Because excessive creation of reactive oxygen species may damage DNA and encourage aberrant cellular growth, oxidative stress and chronic inflammation are thought to be key factors to the development of cancer.^[1] The immune system is essential for preventing the growth of tumors in the body. Natural Killer (NK) cells are a crucial component of the innate immune system and has the capacity to identify and eradicate tumor cells without requiring previous sensitization. By releasing cytotoxic proteins like granzyme and perforin, which cause aberrant cells to undergo apoptosis, NK cells eradicate cancer cells.^[2] Research has shown that human exposure to phytoncides can boost NK cell activity and improve immunological

function. Additionally, these substances have anti-inflammatory and antioxidant qualities that may help prevent cancer.^[3] Alpha-pinene is a promising substance for medicinal uses since it has been shown to have antioxidant, anti-inflammatory, antibacterial, and maybe anticancer properties.^[4] The use of topical herbal formulations, including medicated soaps, to transfer bioactive chemicals via the skin is becoming more and more popular. In addition to serving as a barrier of defense, the skin influences immunological responses. Thus, adding phytoncide chemicals to soap compositions may boost immune activation through skin exposure and offer antioxidant and anti-inflammatory effects.^[5]

1.1 SOAP

In order to maintain personal hygiene and avoid infections, soap is a frequently used washing substance that helps remove dirt, oils, and microorganisms from the skin. The extra health advantages of soaps containing natural bioactive ingredients have drawn attention in recent years. Compounds originating from plants, such

alpha-pinene, have anti-inflammatory, anti-cancer, antioxidant, and perhaps immune-boosting qualities. As a result, adding these substances to soap formulas may offer both purifying and maybe medicinal advantages.

1.2 PHYTONCIDES AND THEIR BIOLOGICAL ACTIVITY

- Plants emit volatile chemical substances called phytoncides to defend against insects, fungus, and bacteria. Boris P. Tokin was the first to use the word "phytoncide".^[6]
- Phytoncides, which include bioactive monoterpenes like limonene, β -pinene, and alpha-pinene, are frequently found in forest trees including cypress, pine, and cedar.^[7]
- By preventing the development of bacteria and fungus, phytoncides demonstrate antibacterial action.^[8]
- These substances also have antioxidant properties, which aid in reducing oxidative stress and neutralizing free radicals.^[7]
- By lowering inflammatory mediators, phytoncides have anti-inflammatory qualities.^[9]
- It has been demonstrated that exposure to phytoncides increases the activity of Natural Killer Cells, which are crucial for eliminating tumor and virus-infected cells.^[10]

1.3 ROLE OF NK CELL IN CANCER PREVENTION

- An integral element of the innate immune system, natural killer cells are critical for identifying and destroying abnormal cells, such as tumor and virus-infected cells.^[11]
- Unlike T-cells, NK cells may eliminate cancer cells without first being sensitized. They use activating and inhibitory receptors to identify stressed or altered cells.^[12]
- NK cells destroy tumor cells by releasing cytotoxic chemicals like granzymes and perforin, causing cancer cells to undergo apoptosis, or programmed cell death.^[13]
- NK cells release immunological-regulating cytokines such as interferon-gamma (IFN- γ), which promotes immune responses and prevents tumor growth.^[12]
- While decreasing NK cell function is connected to increased vulnerability to tumor growth, increased NK cell activity has been linked to a lower risk of cancer.^[14]

2. MATERIALS AND METHODS

Collection of active ingredients were collected from different manufacturing company and local market. Alpha -pinene, lactic acid, guar gum, methyl paraben – SD Chemical, decyl glucoside -BRM Chemicals, Turmeric-local market.

2.1 SOAP INGREDIENT

S.NO	INGREDIENT	USES
1.	Active pharmaceutical ingredient	Anti-cancer, Anti-inflammatory, Anti-oxidant
2.	Methyl paraben	Preservative
3.	Decyl glucoside	Surfactant
4.	Turmeric	Anti-oxidant
5.	Guar gum	Binders
6.	Lactic acid	Buffering agent
7.	Water	Aqueous vehicle
8.	base	humectant

2.2 MELT AND POUR METHOD PROCEDURE

The glycerin soap base was first cut into small pieces and melted using a water bath at controlled temperature to avoid overheating. In a separate container, guar gum was dispersed slowly in the aqueous vehicle with continuous stirring to form a smooth gel without lumps. Turmeric was then added to the gel and mixed uniformly. Decyl glucoside was incorporated into the mixture with gentle stirring to avoid excess foam formation. After uniform mixing, alpha pinene was added dropwise and blended thoroughly to ensure even distribution. The prepared mixture was slowly introduced into the melted glycerin soap base with continuous stirring to obtain a homogeneous formulation. A small quantity of lactic acid was added to adjust the pH and improve stability. The final blend was stirred until smooth and poured into molds, allowed to cool at room temperature, and left undisturbed until solidification. The solidified soap was removed from the molds and stored in airtight containers for further evaluation.



Figure 01: Melt and pour method.

2.3 SOAP FORMULATION WITH DIFFERENT CONCENTRATION

Table 2: soap formulation with different concentration.

INGREDIENT	F1	F2	F3
Base	75gram	75gram	75gram
Active pharmaceutical ingredient	15ml	10ml	5ml
Decyl glucoside	3ml	2.5ml	2ml
Lactic acid	2-3 drops	2-3 drops	2-3 drops
Guar gum	2-3 drops	2-3 drops	2-3 drops
Turmeric	1.5ml	1ml	0.5ml
Methyl paraben	2ml	1.5ml	1ml
Distilled water	q.s	q.s	q.s

3. EVALUTION CRITERIA

3.1 Physical examination

Organoleptic features, such as appearance, color, and odor, were identified.



Figure 02: anti-cancer soap.

3.2 PH

A digital pH meter was used to measure the pH when 1 g of soap was dissolved in 10 mL of distilled water.

3.3 Foam height 25 ml of distilled water were blended with a 0.5-gram sample of soap. Next, transfer it to a 100 ml measuring cylinder and top it off with water to reach 50 ml. During 25 strokes, the foam height was measured above the aqueous volume, and the aqueous volume was measured up to 50 ml.



Figure 03: foam height.

3.4 Foam retention

25 ml of the 1% soap solution was put into a 100 ml graduated measuring cylinder. The measuring cylinder's top was sealed with our hand, and after 4 minutes of agitation, the volume of foam was measured every minute.

3.5 Test for skin irritation

After applying soap to the skin for 10 minutes, the reaction was noted and recorded.

3.6 Total fatty matter

Fatty matter was computed by observing the quantity of fatty acids that were formed after the soap and acid reacted with hot water. Upon dissolving 10 g of soap that was prepared in 150 ml of distilled water, it was then heated. Upon heating this to a clear solution, 20 ml of 15% H₂SO₄ were added. After heating the resulting solution again, 7 g of beeswax is added to harden the surface fatty acids. A cake was formed. The resulting cake was then dried and weighed based on the following formula to determine TFM.

$\% \text{TFM} = \frac{(\text{Weight of the cake} - \text{Weight of the wax})}{\text{Weight of the soap in g}} \times 100$.

3.7 Determination of total moisture content

Total moisture content was calculated by placing 5 g of the soap in a petri dish and drying it for two hours at 105°C in a hot-air oven. After that, it was weighed, heated, and cooled. The moisture content is indicated by the weight difference.^[15]

$\text{Water content} = \frac{m}{M} \times 100$ m = loss in mass of the material after drying M = mass of sample taken.



Figure 04: Total moisture content.

3.8 In Vitro Cell Viability Assay

- A549(Human lung cancer cell line) was procured from national center for cell sciences (NCCS), Pune maintained in DMEM Medium supplemented with 10% fetalbovineserum.

- Cell were incubated at a concentration of 1×10^4 cells/ml in culture medium for 24 h at 37°C and 5%CO₂.
- Cell were seeded at a concentration $(70\mu\text{l})10^4$ cell/well in 100 μl culture medium and 100 μl
- I. Sample (10-100 $\mu\text{l}/\text{ml}$) into microplate respectively (tissue culture grade and 96 wells)
- Control wells were incubated with DMSO (0.2% in PBS) and cell line. all sample were incubated in triplicated. controls were maintained to determine the control cell survival and the percentage of live cells after culture
- Cell culture was incubated for 24h at 37°C and 5% CO₂ in CO₂ incubator (thermos scientificBB150)
- After incubation the medium was completely removed and added 20 μl of MTT reagent (5mg/minPBS)
- After addition of MTT, cell incubated for 4hrs at 37°C in CO₂ incubator.
- Observed the wells for formazan crystal formation under microscope. The Yellowish MTT was reduced to dark colored formazan by viable cells only.
- After removing the medium completely. Added 200 μl of DMSO (kept for 10min) and incubate at 37°C (wrapped with aluminum foil).

Triplicate samples were analyzed by measuring the absorbance of each sample by a Elisa microplate reader (**Bioline 208**) at a wavelength of 570nm.

4. RESULT AND DISCUSSION

S.NO	PARAMETERS	RESULTS
1	Colour	Orange - red
2	Odour	Aromatic
3	Shape	Rectangle
4	pH	8
5	Foam height	4.5 cm
6	Foam retention	5 mins 15 sec
7	Texture	Smooth
8	Washability	Good washability
9	Skin irritation	Non irritant
10	Total moisture content	12.4 percentage
11	Total fatty matter	60 percentage

In Vitro Cell Viability Assay

The cytotoxic activity of the Sample–Soap against the A549 human lung cancer cell line was evaluated using the MTT assay, and the results demonstrated a clear dose-dependent reduction in cell viability. The control group exhibited 100% viability with a mean absorbance of 2.110. Treatment with the sample at 10 $\mu\text{L}/\text{mL}$

reduced cell viability to 72.35%, which further decreased to 58.04% at 40 $\mu\text{L}/\text{mL}$ and 40.44% at 100 $\mu\text{L}/\text{mL}$. The calculated IC₅₀ value of 64.34 $\mu\text{g}/\text{mL}$ indicates that the sample possesses moderate cytotoxic activity against A549 cells.

Table no 03: Effects Of 5-Flurouracil against A549 (Human Lung Cancer Cell line) MTT Assay.

Sr.no	concentration ($\mu\text{g}/\text{ml}$)	Absorbance (O D)				Cell viability (%)	IC50 ($\mu\text{g}/\text{ml}$)
		1	2	3	Average		
1.	Control	2.107	2.114	2.11	2.110		
	Standard-5FU 10	1.112	1.113	1.114	1.113	52.74	7.88 $\mu\text{g}/\text{ml}$
	40	0.422	0.426	0.438	0.428	20.28	
	100	0.329	0.395	0.385	0.369	17.48	

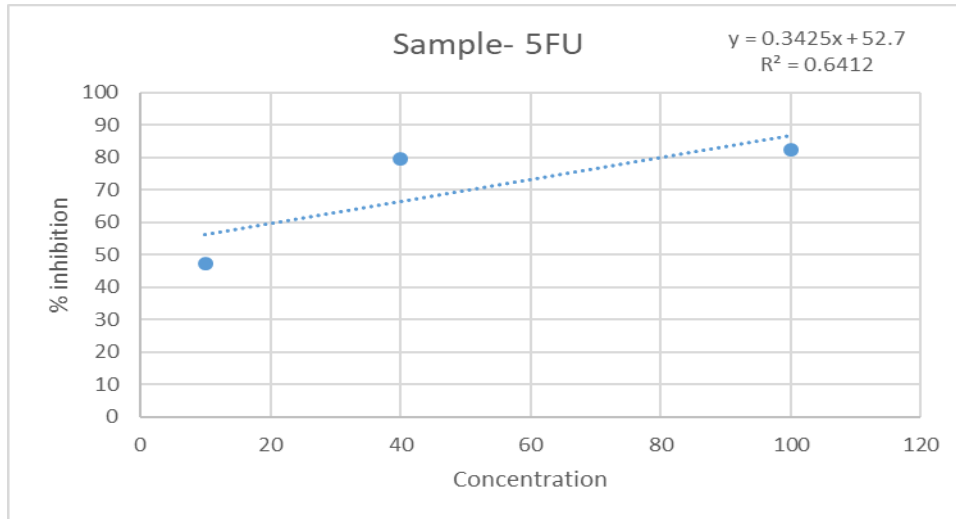


Fig no 05: Graphical Demonstration of sample -5FU.

Table no 04: Effects of Sample against A549 (Human Lung Cancer Cell line) by MTT assay.

Sr.no	concentration (µl/ml)	Absorbance (O D)				Cell viability (%)	IC50 (µg/ml)
		1	2	3	Average		
1.	Control	2.107	2.114	2.11	2.110		
2.	Sample - Soap 10	1.523	1.529	1.528	1.526	72.35	64.34
	40	1.223	1.225	1.226	1.224	58.04	
	100	0.852	0.851	0.857	0.853	40.44	

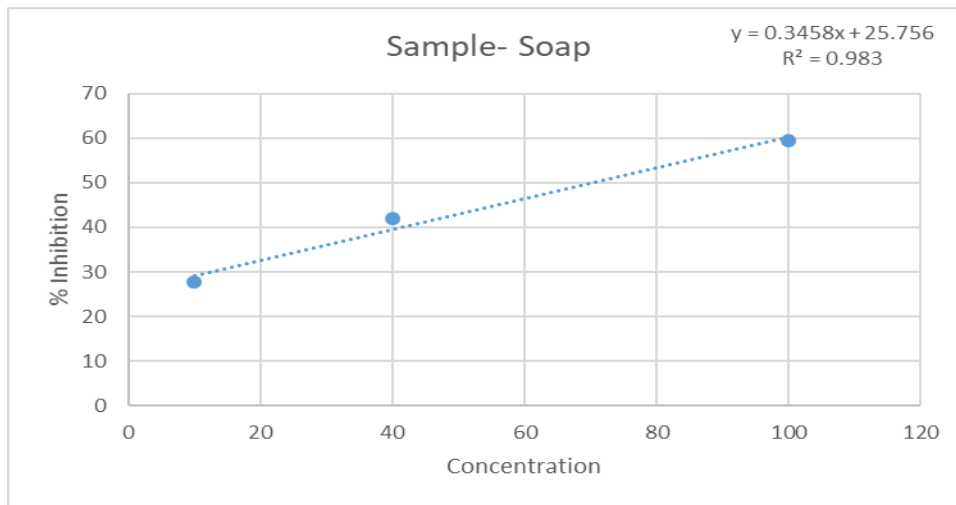
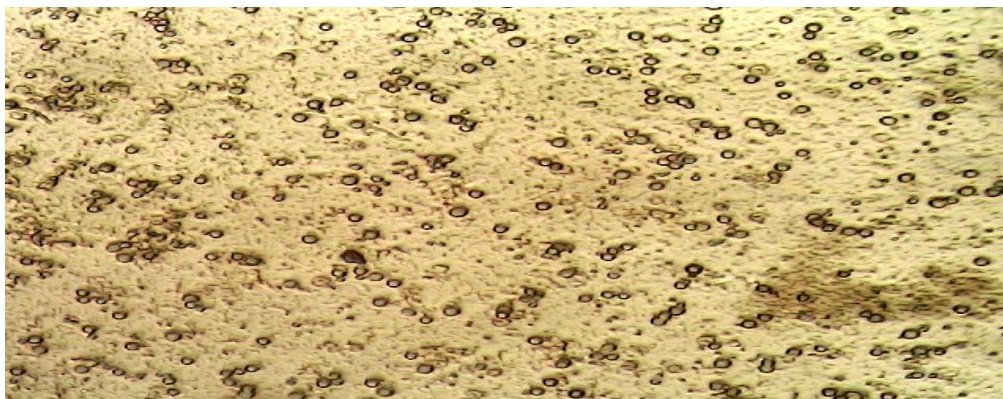
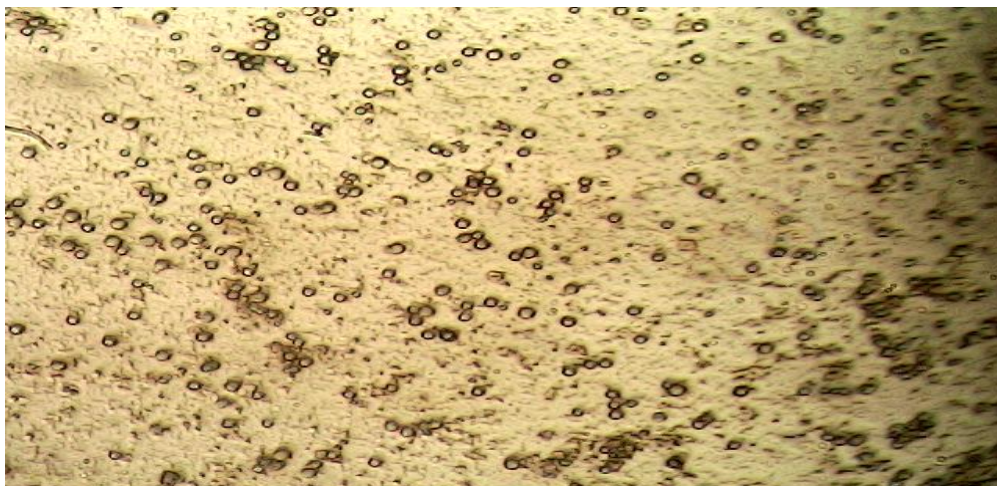


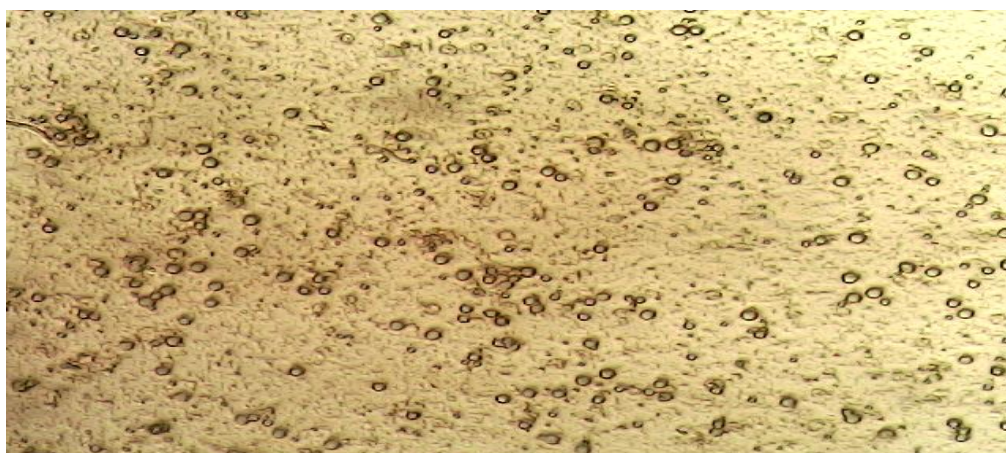
Fig no 06: Graphical Demonstration of sample-soap.



SAMPLE SOAP A549 CELLS LUNG CANCER CELLS



STANDARD 5FLU IN A549 CELLS LUNG CANCER CELLS



CELLS (UNTREATED) IN A549 CELLS LUNG CANCER CELLS

DISCUSSION

Colour, odour, appearance and pH were among the organoleptic properties of soap that were measured. Total fatty matter, moisture content, foam height, and foam retention are among the estimated parameters that are displayed in the table. The soap does not irritate the skin.

The cytotoxic activity of the Sample–Soap against the A549 human lung cancer cell line was evaluated using the MTT assay, and the results demonstrated a clear dose-dependent reduction in cell viability. The control group exhibited 100% viability with a mean absorbance of 2.110. Treatment with the sample at 10 $\mu\text{L}/\text{mL}$ reduced cell viability to 72.35%, which further decreased to 58.04% at 40 $\mu\text{L}/\text{mL}$ and 40.44% at 100 $\mu\text{L}/\text{mL}$. The calculated IC_{50} value of 64.34 $\mu\text{g}/\text{mL}$ indicates that the sample possesses moderate cytotoxic activity against A549 cells.

5. CONCLUSION

The immuno-activating phytoncides soap demonstrated promising anticancer activities in in-vitro studies. The formulation showed dose-dependent cytotoxicity against lung cancer cell lines while enhancing NK cell activation markers, suggesting stimulation of innate immunity. Strong free radical scavenging and inhibition of

inflammatory pathways further support its potential role in cancer prevention.

Overall, this topical phytoncide-based formulation may serve as a supportive preventive strategy by reducing oxidative stress, suppressing chronic inflammation, and enhancing NK cell-mediated immune surveillance. However, further mechanistic studies and clinical evaluations are required to confirm its efficacy and safety in humans.

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