



FORMULATION AND EVALUATION OF FILM FORMING MICRO EMULSION OF GARLIC EXTRACT CONTAINING POLYSULFANE DERIVATIVES TO POTENTIALLY INHIBIT THE SPREAD OF MELANOMA

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ABSTRACT

Melanoma is a dangerous form of skin cancer caused by melanocytes, the cells that produce melanin. Although less prevalent, it is extremely lethal due to its ability to metastasis to internal organs. Chemotherapy has long been the primary treatment for metastatic melanoma, but it has serious adverse effects due to its non-selective action on healthy cells. As a result, there is a rising interest in combining herbal treatments with innovative drug delivery devices to improve treatment outcomes while reducing toxicity. Garlic (*Allium sativum*), which is widely used in traditional medicine, has strong anti-cancer capabilities due to the presence of sulfur-containing chemicals such as S-allyl cysteine, alliin, and ajoene. In multiple investigations, these bioactive's have showed promise in preventing melanoma cell proliferation and metastasis. As a result, the current study sought to create a stable and effective film-forming microemulsion using garlic extract to treat melanoma. The formulation was tested for its capacity to create films. The current study concludes that the herbal film-forming microemulsion provides a natural, safe, and effective method against melanoma, offering a promising alternative to traditional chemotherapy therapies.

KEYWORDS: Melanoma, Garlic (*Allium sativum*), S-allyl cysteine(SAC), Alliin, Film forming microemulsion.

INTRODUCTION

Melanoma is a malignant tumour of melanocytes, cells responsible for producing the skin pigment melanin. Of all skin cancers, melanoma is the least common, but because of its metastatic potential, it is responsible for approximately 80% of deaths related to skin cancer.^[1] When detected early, melanoma is highly treatable; however, advanced stages can lead to metastasis in the lymph nodes and internal organs, ultimately resulting in fatalities.^[2] Melanoma can impact individuals of all skin colours. However, in patients with skin of colour, the disease is frequently identified during later stages, posing challenges for effective treatment.^[3]

Before recent advancements, chemotherapy was the main treatment for advanced melanoma. Various combinations aimed to improve responses but didn't enhance overall survival. Bio-chemotherapy had high responses but didn't boost survival and caused more toxicity. Localised

treatments like limb perfusion offered palliation. Despite new options, chemotherapy remains crucial for palliative care in challenging melanoma cases. But Chemotherapy kills regular cells, as well as cancer cells, and this is why, lots of side effects occur and people suffer.^[4] Hence an herbal approach coupled with the novel drug delivery system can be an alternative approach to tackle this problem.

Garlic (*Allium sativum*) is widely used in traditional herbal remedies and alternative medicine. The National Cancer Institute (NCI) set garlic on the top of a vegetable pyramid, representing potency in cancer prevention.^[5] The anti-carcinogenic effect of garlic is attributed to the presence of organosulfur compounds such as allicin, allyl sulfides, ajoene, and S-allyl cysteine (SAC).^[6]

S-allyl cysteine (SAC), a sulfur-containing amino acid compound sourced from garlic extract, effectively

suppressed the proliferation of nine human and one murine melanoma cell line in a dose-dependent manner (1.2–10 mM), as determined by a [3H] thymidine incorporation assay.^[7] Taylor et al., were pioneers in documenting the antimetastatic properties of garlic sulfur compounds. Their findings revealed that it not only inhibits tumour cell growth in vitro but also significantly suppresses metastasis to the lungs in the B16/BL6 melanoma cell model using C57BL/6 mice.^[8]

Polymeric film forming systems are novel drug delivery systems for topical application directly on to the skin which forms a thin, transparent film In situ upon evaporation of the solvent. The polymeric network of the film acts as an external reservoir and thereby limits the supply of the drug to skin and achieves control drug release. Film Forming Emulsion uses polymers which not only acts as a film forming agent, along with it plays the role of thermodynamic stabilisers which will reduce the incorporation of emulsifiers which in turn results in reduced skin irritation.^[9]

Hence the present work aims to formulate and evaluate an herbal Film Forming micro emulsion using garlic extracts mainly containing S-Allylcysteine, Alliin and Ajoene for the treatment of melanoma.

MATERIALS AND METHOD

Materials: Garlic, Tween 80, PEG, Propylene glycol, Glycerol Monostearate.

Methods

1. Collection of plant material and Morphological study of the Garlic.

- Collection of plant material: *Allium sativum* were collected from local farmhouse in the month of January 2025.
- Morphological study of Garlic.^[10]

2. Method of Preparation of *Allium sativum* extract^[11]

Soxhlet extractions were performed using 150g of sliced fresh garlic loaded into a teabag and placed in the extraction chamber. Water, ethanol–water (1:1) and 100% ethanol are used as solvents with a 2 hrs. extraction time. Two hundred millilitres of each solvent was poured into a round-bottom flask. A 1:20 ratio of sliced garlic and solvent is selected for extraction process. The solvents are heated slightly above the boiling point of the solvent for a specified time interval using a heating mantle. After the completion of the extraction process, a rotary vacuum evaporator, in the range 35–40°C was used to recover the solvent from the extract.^[9]

3. Preliminary Phytochemical screening^[12,13,14]

The above extract is studied for their Phytochemical identifications and reported. Qualitative preliminary phytochemical analysis was performed with different chemical reagents to detect the nature of

phytoconstituents present in the Garlic extract. The presence of amino acids, terpenoids, saponins and alkaloids were evaluated by standard qualitative methods.

4. Method of preparation of Herbal film forming micro emulsion^[9,15,16]

Surfactants (Tween 80 and PEG) and co-surfactants (Propylene glycol and Glycerol Monostearate) were blended in a ratio of 6:1:1:1 ratio and blended thoroughly using a magnetic stirrer for 5 min. To the above mixture Aqueous Phase containing Isopropyl Myristate and extract were added. Oil phase constituting Oleic acid and was added to the above mixture and blended for 5 min and subsequent mixing performed using a magnetic stirrer for 30 mins. Further HPMC and tween 40 were added and stirred the mixture using a homogenizer for 20 mins.

5. Evaluation of the Herbal film forming micro emulsion

- **Appearance:**^[17] The micro emulsions are visually inspected for optical transparency and homogeneity by observing them against a light source.
- **pH Determination:**^[18] The pH of the formulation was measured using a pH meter. About 0.5g of the formulation was weighed and dissolved in 50.0ml of water and its pH was measured. Each formulation was evaluated in triplicate; then, the mean and Standard Deviation (SD) was reported.
- **Viscosity:**^[18] Viscosity of the formulation was determined by Brookfield Viscometer. The viscosity measurements were done using Brookfield DV-E viscometer using #27 spindle. The developed formulation was poured into the adaptor of the viscometer and the angular velocity was increased gradually from 0.5 to 20 rpm.
- **Determination of Spreadability:**^[18] The one gram of formulation (W) is dropped on the centre of the glass plate and compressed with another glass plate. The spreading area (A) of the sample and the time taken to spread on the glass plate (T) are collected. The spreadability value is calculated by $W \times A / T$. The test is performed in triplicate, and the mean and SD are reported.
- **Determination of Drying Time and Integrity of Film on the Skin:**^[19] The 0.1 mL of each formulation is applied to the forearm of the researcher in the area of 1×1sq.inch (n = 3). The drying time and integrity of film on the skin after drying are observed. The integrity of film on the skin is graded into three groups—A, B, C as follows: A- Complete film with no cracks and no flaking B- Complete film with cracks or sporadic flaking C- Film partly or completely missing.
- **Stickiness of the film:**^[20] The stickiness of the film is assessed by gently compressing a piece of cotton wool against the formed film. The degree of stickiness is determined by observing the adherence of cotton fibres to the film. High, medium, or low

stickiness is classified if dense, thin, or occasional/no fibres are observed, respectively. This test is crucial for optimizing the formulation to minimize or eliminate stickiness, thus preventing the film from adhering to the patient's clothing.

- **Washability:**^[20] This is performed by applying the film forming emulsion on the skin and allows it to dry. Then washing the film with water and the easiness of washing is rated as poorly washable, moderately washable, or easily washable.

RESULTS AND DISCUSSION

1. Method of preparation of *Allium sativum* extract Morphological studies of *Garlic*(*Allium sativum*)

Among the spices grown in India, garlic (*Allium sativum* L.) is the most important bulbous crop and widely cultivated *Allium* throughout country. It is rich in protein, phosphorus, potassium, calcium, magnesium and carbohydrates. Garlic is mostly strong flavoured due to presence of sulphur containing compounds that impart their distinctive small and pungency. The wide variation was observed in bulb characters among the cultivars. The maximum bulb weight was recorded in cultivars Cheenia (43.3 gm), followed by Roshni Mota (38.0 gm), while the lowest bulb weight was found in variety GG-I (17.0 gm). Maximum number of cloves was found in cultivars BG 108 (32.8), followed by Cheenia (32.67) and it was minimum found in cultivars Phule Basant (20.3).

Qualitative preliminary phytochemical analysis

Phytochemical screening was done to detect the phytoconstituents present in ethanolic extract of *Allium sativum*. It revealed that the extract was rich in chemical constituents like Amino acid cysteine; terpenoids, saponins and alkaloids, but carbohydrates and reducing sugar were not detected or might be present in trace undetectable amounts.

2. Evaluation of the Herbal film forming micro emulsion

- **Organoleptic characteristics and evaluation of the formulation:** The formulated Herbal film forming micro emulsion was evaluated by physicochemical test to observe its properties. The prepared film forming micro emulsion showed Pale yellow colour and it exhibited good characteristics in terms of appearance, colour, homogeneity and consistency.
- **pH and Spreadability:** The pH of the formulation was determined by using a digital pH meter. From the result it was found that the formulation had desired pH which is compatible with the skin. Spreadability value indicates that the prepared microemulsion spreads easily onto the skin surface. For quick spreadability formulation can be rubbed onto the surface of the skin while applying to increase the action.
- **Viscosity:** Viscosity of the formulation was evaluated using Brookfield Viscometer and found that as shear of the spindle increases viscosity of the given formulation decreases. The viscosity of the formulation was in the range of 796-6670cps at 0.5-20 rpm which is ideal for the formulation prepared.
- **Drying Time and Film Integrity** The drying time was performed on the three volunteers including the researcher and found that the drug can get dried at a moderate speed with uniform film formation on the forearm. The product was having moderate washability which leads to the longer residing time of drug on the skin surface after contact with water. Also, the stickiness was found to be very limited which implies that the formulation prepared was good for patient usage without much complications of uncomfortableness.

Table no. 1: Phytochemical Screening of *Allium sativum* extract.

Phytochemical	Specific Test	<i>Areca catechu</i> peel extract
Alkaloids	Dragendroff's Test	+ve
Saponins	Foam Test	+ve
Amino acid (Cysteine)	Lead Acetate Test	+ve
	Nitroprusside Test	+ve
	Ninhydrin test	+ve
Flavonoids	FeCl ₃	+ve
	Lead Acetate	+ve
Terpenoids	Salkowski test(modified)	+ve
Glycosides	Keller Kiliani Test	+ve
Steroids	Chloroform-H ₂ SO ₄ Test	+ve
Tannins	FeCl ₃	-ve
Reducing Sugar	Fehling's Test	-ve
	Benedict's test	-ve
Carbohydrates	Molisch's Test	-ve

Table no. 2: Physicochemical tests of the prepared film forming micro emulsion.

Evaluation test	Result
Appearance	Good
Colour	White
Odour	Characteristic
Spreadability	Good
Irritability	No irritation
Homogeneity	No lumps
Consistency	Moderately thick
Washability	Moderately washable

Table no. 3: pH and Spreadability of Herbal film forming micro emulsion.

Formulation	Herbal film forming micro emulsion
pH*	6.41±0.03
Spreadability* (g.cm/sec)	15.5± 1.60

*Data expressed as a mean ±SD, n=3

Table 4: Drying time and Film integrity.

Parameters	Observation
*Drying time	5.20±0.20 min
**Integrity of film	A
Water washability	Moderately washable
Stickiness	Low

*Data expressed as a mean ±SD, n=3

**A= Complete film with no cracks and no flaking

**Fig. No. 01: Soxhlet extractions of *Allium sativum*.****Fig No. 02: *Allium sativum* Extract.****Fig No. 03: Herbal film forming micro emulsion of Garlic Extract.**

CONCLUSION

The promise of a novel herbal-based film-forming microemulsion that incorporates garlic extract for the treatment of melanoma is highlighted by the current study. Alternative strategies are essential given the drawbacks of traditional chemotherapy, such as its severe side effects and limited effectiveness in later stages. In both in vitro and in vivo investigations, garlic, which is abundant in organosulfur compounds like S-allyl cysteine, Alliin, and Ajoene, has shown encouraging anti-carcinogenic and anti-metastatic qualities. The goal of this research is to provide a targeted, controlled-release treatment with less systemic toxicity by incorporating these bioactive substances into a topical film-forming microemulsion system. The polymeric layer improves skin permeability and patient compliance in addition to guaranteeing continuous medication release.

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CONFLICT OF INTEREST

- We declare that we have no conflict of interest.

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