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Research Article

EVALUATION OF MELANIN INHIBITORY PROPERTIES OF *KUMKUMADI OIL* AGAINST FORSKOLIN INDUCED MELANIN SYNTHESIS IN A375 CELL LINE

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ABSTRACT

Melasma is a chronic, acquired pigmentary condition characterized by symmetrical, blotchy, brownish facial pigmentation, primarily affecting women. Though not medically harmful, it significantly impacts quality of life, self-esteem, and psychosocial wellbeing. Conventional depigmenting agents such as hydroquinone, corticosteroids, and chemical peels are associated with adverse effects and limited long-term efficacy, thereby necessitating the exploration of safe and natural alternatives. *Kumkumadi Oil*, a classical Ayurvedic polyherbal formulation, has traditionally been employed for improving skin complexion and treating hyper pigmentary conditions. The study investigated the melanin inhibitory potential of *Kumkumadi Oil* against forskolin-induced melanogenesis in A375 melanoma cells and was assessed by MTT assay to determine safe concentrations. Forskolin was used to stimulate intracellular cAMP signaling and enhance melanin production. A375 cells were treated with *Kumkumadi Oil* at 250µg/mL and 500µg/mL, and intracellular melanin levels were quantified spectrophotometrically. Effects were compared with forskolin-induced untreated controls. Results demonstrated a dose-dependent reduction in melanin content, accompanied by significant inhibition of tyrosinase activity, without inducing cytotoxic effects on the tested cells. The study provides *in vitro* evidence that *Kumkumadi Oil* reduces forskolin-induced melanogenesis in human melanoma cells. The present conceptualization establishes a scientific basis for evaluating the efficacy of traditional *Kumkumadi Oil* in melasma and other hyper pigmentary conditions, highlighting its potential to surpass conventional synthetic chemical medicines as a safer and more sustainable therapeutic option.

INTRODUCTION

Vyanga (Melasma) is described as a condition under *Kshudraroga*. It holds significant clinical and cosmetic relevance due to its psychosocial implications.

Clinically, *Vyanga* manifests as dark, hyperpigmented patches, predominantly distributed over the cheeks, nose, forehead, and chin. Its *Nidana* (etiological factors) include vitiation of *Pitta* and *Vata doshas*, often exacerbated by *Manasika* (psychological stressors) such as *Krodha* (anger), *Shoka* (grief), and *Ayasa* (mental stress).

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The cardinal symptoms of *Vyanga* include *Niruja* (painlessness), *Tanu* (thin patches), and *Shayava varna mandala* (bluish-black pigmented macules). [1]

In contemporary systems of medicine, Melasma is a chronic, acquired pigmentary disorder predominantly influenced by ultraviolet (UV) radiation, as well as hormonal and genetic factors. Pigmentation results from excess melanin production by melanocytes. It presents as brown macules or irregular patches with symmetrical distribution, commonly affecting the forehead, upper lip, and cheeks. [2] Although melasma is not life-threatening, its persistent nature, cosmetic impact, and high recurrence rate render it a considerable therapeutic challenge in dermatological practice.

The treatment and management of *Vyanga* in Ayurveda emphasize on pacification of the vitiated *Doshas*, systemic detoxification, and the use of local

applications aimed at restoring complexion and maintaining skin balance.

Kumkumadi Oil is a classical Ayurvedic formulation described in authoritative compendia such as *Ashtanga Hridaya, Bhaishajya Ratnavali, Yogaratnakara,* etc., It is specifically known for its *Varna-prasadana* (enhancing skin complexion), *Tvak-prasadana* (alleviating blemishes), and *Vayasthapana* (maintaining youthful skin) actions.

In comparison to conventional therapies such as hydroquinone, corticosteroids, chemical peels, and laser treatments, *Kumkumadi Oil* offers the advantage of being natural, multi-targeted, and traditionally regarded as safe when used appropriately.

The name *Kumkumadi* is derived from its principal ingredient, *Kumkuma* (Saffron, *Crocus sativus* Linn.), combined with the suffix-adi (meaning others), indicating its formulation with saffron as the key component along with other herbs.

In *Kumkumadi Oil*, saffron is complemented by a range of other herbs with similar depigmenting, complexion-enhancing and restorative qualities, creating a synergistic effect. Together, these ingredients make *Kumkumadi Oil* a robust skin formulation, widely used for promoting skin health, reducing pigmentation, and enhancing overall complexion.

This golden glow oil is prepared through the classical Ayurvedic pharmaceutical process of *Taila Paka*, wherein a *Kvatha* (decoction) of selected drugs, a fine *Kalka* (bolus of herbal paste) and *Tila taila* (sesame oil) serve as the primary components. This multi-step preparation ensures the extraction and stabilization of both water-soluble and fat-soluble phytoconstituents, contributing to the formulation's broad therapeutic spectrum. Along with Saffron, the oil contains *Chandana (Santalum album)*, *Manjishta* (*Rubia cordifolia), Padmaka* (*Nelumbo nucifera*), *Ushira* (*Vetiveria zizanioide*s), *Rakta Chandana (Pterocarpus santalinus*) and several other drugs, which synergistically impart complexion-enhancing, anti-inflammatory, anti-oxidant and anti-aging benefits. [3]

METHOD [5] [6] [7]

Outline of the method

The *In vitro* cytotoxicity was performed to find a non-toxic concentration of the *Kumkumadi oil* on A375 cell line by MTT assay. The percentage inhibition of melanin content was examined in A375 cell line by *in vitro* melanin inhibition assay.

Preparation of *Kumkumadi Oil*

10mg of each *Kumkumadi oil* were weighed and dissolved in DMEM-HG medium supplemented with 2% inactivated FBS to obtain a stock solution of 10mg/mL. Further, serial two-fold dilutions were prepared from the stock solution to obtain lower concentrations for cytotoxicity testing. The two non-toxic concentrations were prepared from the stock solution for anti-pigmentation studies.

1Cell line and culture medium

A375 cell line was procured from NCCS, Pune, India. Stock cells were cultured in MEM supplemented with 10% inactivated FBS, penicillin (100IU/mL), streptomycin (100µg/mL) and amphotericin B (5µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25cm^2 culture flasks. Cytotoxicity study was carried out in 96 well microtiter plate and Anti-pigmentation study was carried out in 6 well microtiter plate.

Cytotoxicity study by MTT assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 100,000 cells/mL using MEM containing 10% FBS. To each well of the 96 well microtiter plate, 0.1mL of the diluted cell suspension was added. After 24hrs, when a partial monolayer was formed, the supernatant was gently flicked off, the monolayer was washed once with PBS and different test concentrations were added on to the partial monolayer in the microtiter plates. The untreated cells were maintained as cell control for comparison. The plates were then incubated at 37°C for 24hrs in 5% CO_2 atmosphere and microscopic examination was carried out and observations were noted after 24hrs. The test solutions in the wells were discarded and 100µL of MTT was added with DPBS to each well. The plates were gently shaken and incubated for 3hrs at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100µL of DMSO was added. The plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a micro-plate reader at a wave length of 570nm.

In vitro Melanin inhibition assay

Initially, the cytotoxicity study in A375 cells were carried out by standard method to determine CTC_{50} value. For inhibitory assay, 1.5 x 10^5 cells were seeded in 6 well plates. After reaching 70-80% confluence, the melanin inhibitory study was initiated. The cells were treated with non-toxic test concentrations of *Kumkumadi Oil* and with Forskolin (125µM) for 24 hrs. At the end of incubation period, the supernatants were aspirated from wells and cultures were washed twice with DPBS. The cells were harvested by trypsinization and cell suspension was centrifuged at 2500rpm for 5min. The cell pellet was washed with DPBS, centrifuged and cells were lysed in 400µL of 0.1N NaOH containing 10% DMSO. Samples were heated at a temperature of 60°C for 1hr and

cooled, the absorbance of the cell lysates were measured at 490nm.

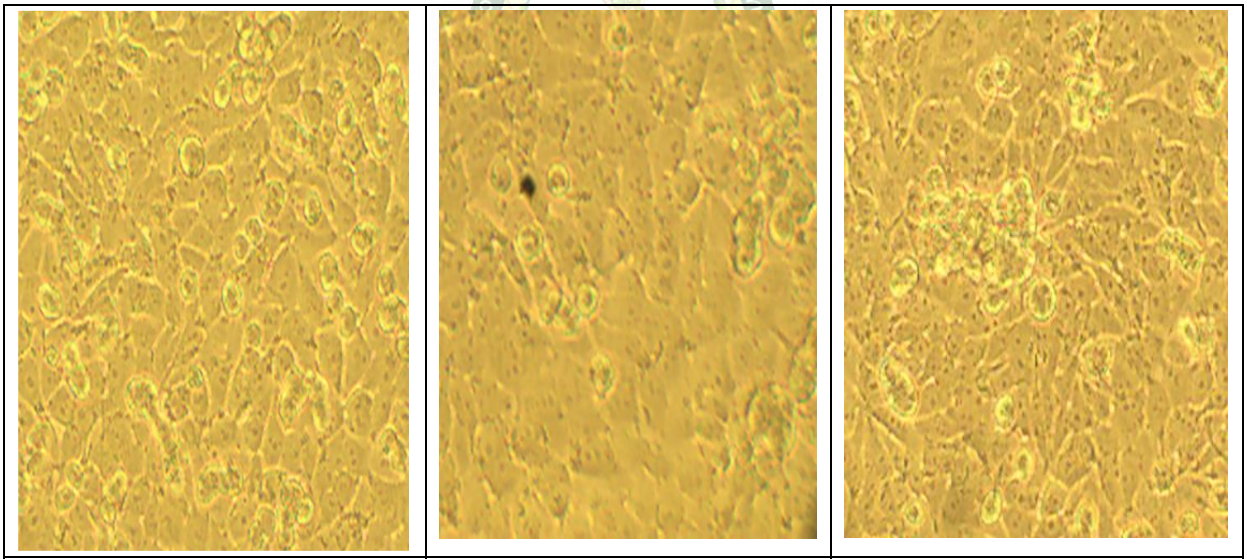
RESULTS

Kumkumadi oil was evaluated for cytotoxicity on the A375 melanoma cell line using the MTT assay.

The IC_{50} value was found to be above 1000µg/mL, indicating minimal cytotoxicity. Two non-toxic concentrations (500µg/mL and 250µg/mL) were selected for subsequent assessment of melanin inhibition. Melanogenesis was induced using forskolin, an activator of adenylyl cyclase that elevates intracellular cAMP levels. Treatment with *Kumkumadi oil* resulted in 40.37% and 35.09% inhibition of melanin synthesis at 500µg/mL and 250µg/mL, respectively. These results indicate that *Kumkumadi oil* effectively suppresses forskolin-induced melanogenesis, potentially via inhibition of adenylyl cyclase and reduction of intracellular cAMP levels.

Table 1: *In vitro* cytotoxicity of *Kumkumadi Oil* in terms of percentage cell viability on A375 cell line by MTT assay (24 hours)

Concentration (µg/mL)	Percentage of cell viability after treatments (Mean ± SD)	CTC_{50} (µg/mL)
1000	72.42 ± 1.30	1429.076
500	78.65 ± 0.42	
250	81.12 ± 1.79	
125	83.78 ± 0.59	
62.5	88.09 ± 1.00	
31.25	91.22 ± 1.45	
15.625	96.68 ± 0.41	
7.8	99.17 ± 0.63	



Control	*Kumkumadi Oil* (1000µg/mL)	*Kumkumadi Oil* (500µg/mL)

Fig.1: Microscopic images showing cell cytotoxicity at 24hrs in cells treated with *Kumkumadi Oil* compared to untreated control on A375 cell line

Table 2: Melanin inhibition percentage of *Kumkumadi Oil* against Forskolin induced melanin synthesis in A375 cell line (24 hours)

S.No.	Test item	Test Concentration	% Melanin Inhibition
1	Forskolin	125µM /mL	--
2	*Kumkumadi Oil* + Forskolin	500µg/mL	40.37 ± 2.29
		250µg/mL	35.09 ± 0.69
3	Vitamin C + Forskolin	100µM/mL	42.43 ± 1.15

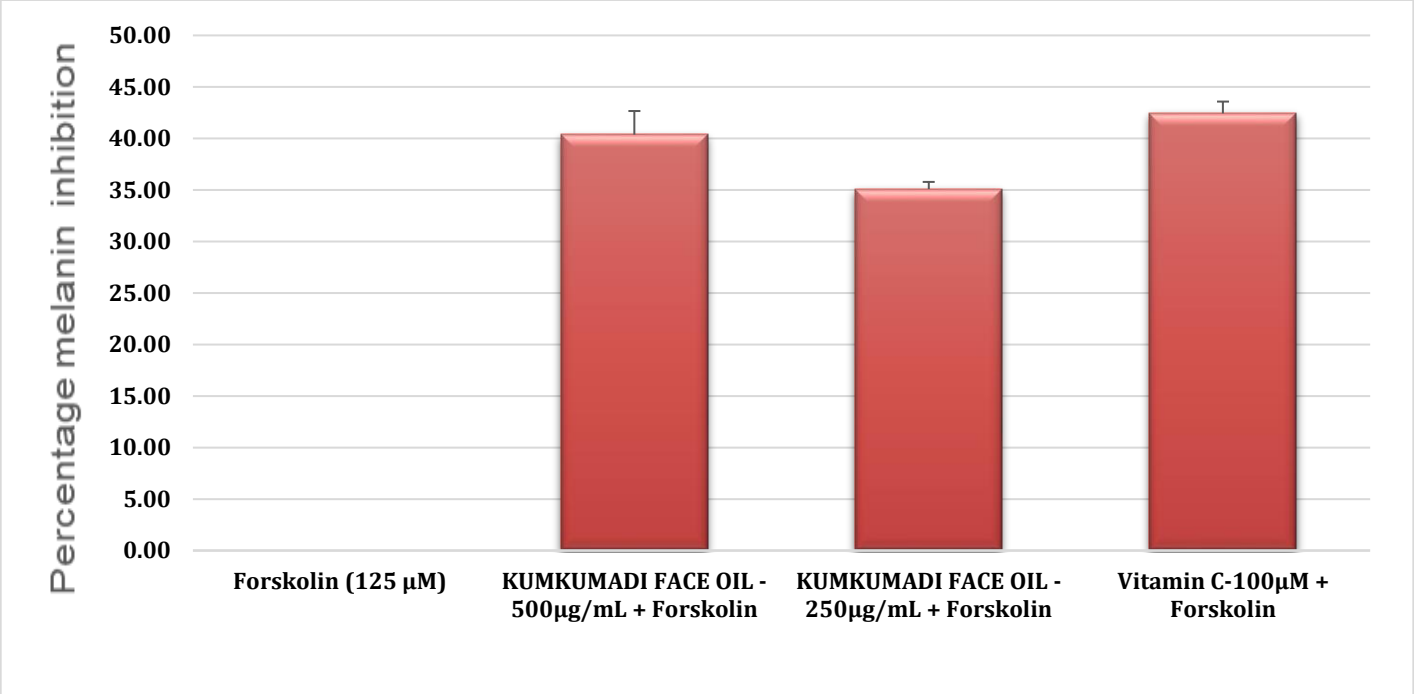


Fig.2: Melanin inhibition percentage of *Kumkumadi Oil* against Forskolin induced melanin synthesis in A375 cell line

DISCUSSION

Understanding *Rasa-Panchakas* of *Kumkumadi Oil*

Predominantly, *Kumkumadi Oil* has *Kashaya* (astringent) - *Tikta* (bitter) - *Madhura* (sweet) *Rasa*.

Kashaya Rasa facilitates *Asra Vishodhana* (blood-purifier) and *Ropana* (wound-healer). The inherent astringent property helps with antioxidant and anti-inflammatory actions that promote tissue repair, reduce post-inflammatory hyperpigmentation, and support collagen stabilization. These attributes help restore skin pigmentation affected by tanning and acne blemishes, while also maintaining skin firmness and elasticity.

Tikta Rasa is known to pacify *Daha* (burning sensation) and *Kandu* (pruritus), while also exhibiting an anti-diaphoretic effect. The bitterness of the *Kumkumadi Oil* aids to its anti-inflammatory, anti-pruritic, and sweat-regulating properties, which help reduce skin irritation and excessive perspiration.

Madhura Rasa possesses *Bala varnakara* (enhancing glow and complexion), *Twachya* (skin-nourishing and protective), and *Vayahsthapana* (anti-aging) properties. Thus, these attributes may be correlated to its anti-oxidant, moisturizing, and rejuvenating effects, which support collagen synthesis, improve skin barrier function, delay cellular senescence, and thereby promote skin health and youthful appearance.

Kumkumadi Oil, characterized by its *Laghu* (light) and *Snigdha* (unctuous) *Gunas*/properties, has been shown to promote *Srotoshodhana* (cleansing of microchannels) and *Dhatu Poshana* (tissue nourishment). These effects contribute to enhanced *Varna* (complexion), support cellular regeneration, and improve *Prabha* (skin radiance).

Kumkumadi Oil, characterized by its *Laghu* (light) and *Snigdha* (unctuous) properties, aids in *Srotoshodhana* (cleansing of microchannels) and *Dhatu Poshana* (tissue nourishment), contributing to enhanced *Varna* (complexion), cellular regeneration, and *Prabha* (skin radiance). This helps to improve dermal penetration and absorption of the oil, thereby enhancing the delivery of active constituents and further promoting skin nourishment and radiance.

Additionally, the *Sheeta Virya* (cooling potency) of *Kumkumadi* Oil supports *Shonitha Prasadana* (blood-purifying) activity, which may underlie its beneficial effects on tissue health and overall skin quality.

Kumkumadi Oil acts through *Pitta* and *Vata dosha* pacification, *Raktashodhana* (blood purification), and nourishment of the *Tvak dhatu*. Classical texts emphasize its role in correcting pathological processes underlying *Tvak vikara* (skin disorders), thereby restoring physiological homeostasis and promoting natural radiance. [8]

Pharmacological actions of the ingredients in *Kumkumadi Oil*

From a pharmacological standpoint, the bioactive constituents of the formulation demonstrate mechanistic relevance to the complex pathophysiology

of melasma. Several components exhibit tyrosinase-inhibitory and melanogenesis-regulatory effects, thereby attenuating hyperactive melanin synthesis. In parallel, their anti-inflammatory and detoxifying actions contribute to the suppression of inflammatory mediators implicated in pigmentary dysregulation. Furthermore, the collective antioxidant capacity of the formulation mitigates oxidative stress, a well-established trigger in the onset and persistence of melasma. [9] Saffron, the lead constituent, has *Varnya* (complexion enhancing) activity in *Vyanga* (melasma). It contains over 160 volatile and non-volatile compounds that has been demonstrated to inhibit melanogenesis by modulating tyrosinase activity and downregulating pathways involving melanin synthesis. [10] The phytochemical compounds in *Yastimadhu* have shown tyrosinase inhibitory activity, and are used in the treatment of hyperpigmentation conditions. [11] *Manjishta,* has *Rakta shodhaka* (blood purifying), *Varnya* (complexion enhancing) properties which shows its significant usage in treating melasma and other pigmentary conditions. [12] The two hydroxyl groups present in Santalin from *Rakta Chandana* contribute to the regulation of melanogenesis through their tyrosinase inhibition activity. [13]

The other ingredients which show their synergistic action together along with the above-mentioned drugs are from *Varnya*/*Lodhradi gana*. These drugs offer a conservative approach in treating skin hyperpigmentation. Their all-encompassing approach in treating inflammation, pigmentation along with their blood-purifying and anti-oxidant actions make *Kumkumadi Oil* a multi-faceted, versatile option in the market. [14] These convergent pharmacological activities offer a coherent scientific basis supporting its therapeutic potential in melasma management.

CONCLUSION

Kumkumadi oil was evaluated for cytotoxicity on the A375 melanoma cell line using the MTT assay. The IC_{50} value was found to be above 1000µg/mL, indicating minimal cytotoxicity. Two non-toxic concentrations (500µg/mL and 250µg/mL) were selected for subsequent assessment of melanin inhibition. Melanogenesis was induced using forskolin, an activator of adenylyl cyclase that elevates intracellular cAMP levels. Treatment with *Kumkumadi oil* resulted in 40.37% and 35.09% inhibition of melanin synthesis at 500µg/mL and 250µg/mL, respectively. These results indicate that *Kumkumadi oil* effectively suppresses forskolin-induced melanogenesis, potentially via inhibition of adenylyl cyclase and reduction of intracellular cAMP levels.

Although conventional melasma therapies such as hydroquinone, retinoids, corticosteroids, and chemical peels demonstrate proven efficacy, their use is frequently constrained by adverse effects, rebound pigmentation, and poor patient adherence. *Kumkumadi taila*, by contrast, provides a holistic intervention with antioxidant, anti-inflammatory, and skin-brightening properties, coupled with a favorable safety profile. Its natural formulation reduces irritation and photo-sensitivity, enabling long-term application without dependency. Beyond addressing pigmentation, it supports overall skin health, thereby emerging as a safer, sustainable, and potentially superior alternative to conventional modalities.

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