

Extract combinations of *Curcuma zedoaria* and *Aloe vera* inhibit melanin synthesis and dendrite formation in murine melanoma cells

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Summary

The objective of the study was to determine the effect of role of combination of extracts of *Curcuma zedoaria* and *Aloe vera* in reducing melanin synthesis. Varying concentrations of the extracts of the plants were tested for melanogenesis and tyrosinase activity in murine melanoma cells.

Extract combinations at a concentration of 1-5 μ l showed 50-150% reduction in melanogenesis without altering the cell proliferation. Tyrosinase activity was very low in extract treated cells when compared to control.

Riassunto

L'obiettivo dello studio è stato quello di determinare il ruolo svolto dall'uso combinato degli estratti di *Curcuma zedoaria* e dell'*Aloe vera* nell'opporsi alla sintesi della melanina.

A questo scopo sono state controllate l'azione di diverse concentrazioni degli estratti vegetali sulla melanogenesi, in particolare sulla tirosinasi, utilizzando culture di cellule melanomiche murine.

L'azione degli estratti in concentrazione da 1 a 5 μ l hanno dimostrato di ridurre il processo melanogenico dal 50 al 150% senza alterare la proliferazione cellulare.

L'attività sulla tirosinasi si è dimostrata molto bassa nelle cellule trattate se paragonata al controllo.

INTRODUCTION

Tyrosinase enzyme plays a major role in melanogenesis process. This rate limiting enzyme oxidizes the amino acid tyrosine to DOPA and then to melanin (1). Retarding the tyrosinase enzyme activity is considered to be the key approach for achieving skin lightening effect with most of the cosmetic skin lightening/whitening/fairness creams (2, 3). Further, this method of inhibiting the melanogenesis process is reversible in nature hence does not produce any permanent pigmented problem to the skin.

Melanocytes are dendritic cells and the transfer of melanosomes to the keratinocytes is aided by the dendrites in the melanocytes (4).

Qualitative and or quantitative changes to the dendrites in the melanocytes play a major role in the melanization of the skin. Dendrite modification is therefore inevitable for the regulation of skin colour besides modulating tyrosinase activity and melanin synthesis within the melanocytes. In the systems of Indian medicine, particularly in Siddha system, several plants have been recognized to have effect in modulating the skin pigmentation.

The glabradin, isolated from licorice, arbutin from mulberry and some other compounds of natural origin have been widely used in several skin lightening preparations all over the world. *Curcuma zedoaria* and *Aloe vera*, although used extensively in various cosmetic and drug preparations, the effect of the combinations of these extracts in inhibiting melanin synthesis is not studied in detail.

In the present paper, we discuss the role of the combination of extracts of *Curcuma zedoaria* and *Aloe vera* in reducing the melanogenesis through tyrosinase inhibition and along with their effect in modulating dendricity pattern in melanocytes.

MATERIALS AND METHODS

The rhizomes of *Curcuma zedoaria* and leaves of *Aloe vera* were extracted separately in propylene glycol: water at 1:1 ratio. The solid to liquid ratio was maintained at 1:100. The extracts were combined at 1:1 ratio.

The mixture of the extract at varying concentrations were used for testing the activity.

Cell Culture

B16F10 murine melanoma cells were cultured in Eagles minimal essential medium supplemented with 10% heat inactivated fetal bovine serum and 2mM L-glutamine at 37°C in a humidified atmosphere containing 5% CO₂.

Different concentrations of the extract ranging from 1-5 μ l were added to the culture after the cells being seeded.

The cells were incubated for 24, 48 or 72 hrs and cell numbers (determined by counting in a haemocytometer chamber), melanin contents and tyrosinase activities were determined in triplicate for each treatment as detailed below.

Melanin Measurement

Melanin content was measured as per the method described as follows. Approximately 10⁷ cells were pelleted by centrifugation at 1000 g for 5 minutes and then washed twice with phosphate buffered saline.

After further centrifugation, the supernatant was decanted, the precipitated cells were re suspended in 200 μ l of distilled water, and 1 ml of ethanol-ether 1:1 was added to remove opaque substances other than melanin.

The mixture was stored and suspended at room temperature for 15 minutes.

After further centrifugation at 3000 g for 5 minutes, the precipitate was solubilized by treatment with 1 ml 1N NaOH/10% dimethyl sulfoxide at

80°C for 30 minutes in a capped tube. The absorbance was measured at 400 nm and the melanin content per cell was calculated and expressed as percentage of control (=100%).

Tyrosinase Assay

Tyrosinase activity was assayed as DOPA oxidase activity.

Approximately 10^7 cells were pelleted and then washed twice with phosphate buffered saline. After centrifugation, the supernatant was decanted.

The cell pellet was dissolved in 1.0 ml of 0.5% sodium deoxycholate in distilled water and allowed to stand at 0°C for 15 minutes.

Tyrosinase activity was assayed spectrophotometrically by following the oxidation of DOPA to dopachrome at 475 nm. The reaction mixture consisting of 3 ml of 0.1% L-DOPA in 0.1 M phosphate buffer, pH6.8 was mixed with cell lysate.

Assay was performed at 37°C in a spectrophotometer. The reaction rate was measured during the first 10 minutes of the reaction while it was linear.

Corrections for auto oxidation of L DOPA in controls were made.

Specific activity was defined as the amount of DOPAchrome formed per 10 min per cell, and is expressed as percentage control (=100).

Dendrite length and number measurement

The extracts treated melanocytes were examined under microscope and the number and relative length of dendrites in each melanocytes were recorded at random and compared with untreated control.

RESULTS

Reduction in melanin content was observed in cell pellets incubated with the extract. However, the growth rate of B16F10 cells was not significantly altered by the extract treatment during 72 hr incubation period, both with controls and extract treatment.

This clearly indicates that the melanogenesis modulation occurs in the cells without affecting the cell proliferation. The level of decrease of melanin synthesis in relation to the concentration of the extracts was 50% to 90% for 1-5 μ l of the extract combinations respectively (Table I).

TABLE I
Melanin synthesis inhibition activity

Extract combination	Melanin inhibition (%)
1 μ l	50
2 μ l	61
3 μ l	78
4 μ l	88
5 μ l	90

Optimum concentration of the extract that showed very high activity in decreasing the melanin synthesis was 4 μ l. Higher than this level did not significantly retard the melanogenesis in the murine B16F10 cells.

The tyrosinase activity was recorded to be very low in cells treated by the extract combination when compared to control (Table II).

The extract up to a concentration of 50 μ l did not show cytotoxicity when tested by MTT.

The number of dendrites in treated melanocytes were significantly lower when compared to the untreated control.

A total of ± 15 dendrites were recorded in melanocytes under control group whereas the number of dendrites recorded in the treatment group was ± 6 .

Further, the length of the dendrites have reduced from 112 μ m length in the case of control to 64 μ m in the treatment group (Table III).

TABLE II

Tyrosinase inhibition activity

Extract combination	Tyrosinase inhibition (%)
1 μ l	28
2 μ l	37
3 μ l	44
4 μ l	67
5 μ l	72

TABLE III

Dendricity modulation - DN= Dendrite Number - DL= Dendrite Length

Study details	Control		Test	
	DL /micron	DN	DL/micron	DN
Control	15 \pm 3	112 \pm 6	-	-
Test (Con.in μ l)				
1	-	-	100 \pm 4	14 \pm 3
2	-	-	95 \pm 5	12 \pm 2
3	-	-	83 \pm 6	10 \pm 4
4	-	-	71 \pm 4	8 \pm 2
5	-	-	64 \pm 6	6 \pm 2

Data expressed as Mean \pm SD

DISCUSSION

The present investigation clearly suggests that the combination of the extracts of *Curcuma zedoaria* and *Aloe vera* popularly referred as 'Evefresh' is very effective in decreasing the melanin synthesis when tested in murine melanoma cells.

The key mechanism of action in the extract combination was found to be through tyrosinase inhibition.

Tyrosinase, being the primary enzyme that plays a major role in the oxidization of tyrosine to melanin, most of the approaches for achieving the skin lightening effects through various cosmetic preparations were by inactivation of the above enzyme. Further, the above approach is reversible, which is very safe and does not cause any permanent damage.

However, melanin transfer inhibitors are also widely used for the above purpose. The melanin transfer inhibitors may or may not interfere in the melanogenesis but by their melanin transfer inhibition mechanism can significantly influence the skin pigmentation. In the present study, we have observed that combination of extracts of *Curcuma zedoaria* and *Aloe vera* possess dual mechanism of down regulating the melanin synthesis and its further transfer to the skin.

This is a unique phenomenon and was not reported or known.

Curcuma zedoaria, being a very old traditional plant used in India for skin care is also known to have antiseptic properties (5-7).

Similarly, *Aloe vera*, the other widely used medicinal plant for various cosmetic and drug preparations all over the world.

This plant is also known to have suncreening effect and hence the use of this plant extract in skin creams offers sun protection benefit (8).

The potent skin lightening effect what we established for the combination of the extracts of *Curcuma zedoaria* and *Aloe vera* assumes great

importance as both plants are widely used for various skin benefits.

Retarding the melanogenesis known to make the skin relatively more vulnerable to UV damage, and that is why most of the skin lightening creams contains sun screeners. Use of the combination of the above extracts offers dual benefit of skin lightening effect as well as sun protection to the skin.

This approach also would provide the advantage of eliminating the chemical sunscreener in the product, thereby can ensure total benefit come purely from herbal source.

This is the first report to our knowledge that the combinations of extracts of *Curcuma zedoaria* and *Aloe vera* having the dual inevitable property of inhibiting the melanin synthesis and also act as melanin transfer inhibitor to the keratinocytes.

The above findings validates the astute science of the ancient Siddha system of medicine as the formulation and its benefits are well documented in the literature of Siddha system of India but the exact mechanism of action was not known.

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